



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07D 487/04, 491/048 A61K 31/40 // (C07D 487/04 C07D 209:00, 209:00) (C07D 491/048, 307:00, 209:00)	A1	(11) International Publication Number: WO 93/06105 (43) International Publication Date: 1 April 1993 (01.04.93)
(21) International Application Number: PCT/US92/08228 (22) International Filing Date: 28 September 1992 (28.09.92)		(74) Agent: PRICE, Robert, L.; Lowe, Price, LeBlanc & Becker, 99 Canal Center Plaza, Suite 300, Alexandria, VA 22314 (US).
(30) Priority data: 765,746 26 September 1991 (26.09.91) US 861,329 31 March 1992 (31.03.92) US		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).
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(54) Title: SUBSTITUTED PHENSERINES AND PHENYLCARBAMATES OF (-)-ESEROLINE, (-)-N1-NORESEROLINE, AND (-)-N1-BENZYLNORESEROLINE; AS SPECIFIC INHIBITORS OF ACETYLCHOLINESTERASE		
<p style="text-align: right;">(I)</p>		
(57) Abstract	<p>The present invention relates to substituted phenylcarbamate or naphthylcarbamate tricyclic compounds of formula (I), wherein R is -O- or NR₁, which provide highly potent and selective cholinergic agonist and blocking activity and their use as pharmaceutical agents. The invention further relates to improvements in therapy relative to cholinergic diseases such as glaucoma, Myasthenia Gravis, Alzheimer's disease and to improvements in therapy and organophosphate poisoning. The invention further provides for a selective acetylcholinesterase and butyrylcholinesterase agents and a method for inhibiting these esterases.</p>	

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SUBSTITUTED PHENSERINES AND PHENYLCARBAMATES OF
(-)-ESEROLINE, (-)-N1-NORESEROLINE, AND
(-)-N1-BENZYLNORESEROLINE; AS SPECIFIC INHIBITORS OF
ACETYLCHOLINESTERASE

Technical Field

The present invention relates to improvements in the treatment of diseases, and more particularly to compounds which exhibit selective inhibition of acetylcholinesterase and butyrylcholinesterase.

Background Art

Physostigmine, also called eserine, and particular derivatives of physostigmine are anti-cholinesterase inhibitors which are well known. Such well known compounds are also useful in the treatment of glaucoma, Myasthenia Gravis, Alzheimer's disease and as antidotes against poisoning with organophosphates.

Physostigmine was introduced into England in 1840 by Daniell (a British medical officer) in the form of the Calabar bean. The compound itself was first isolated by Jobst and Hesse in 1864. Physostigmine has been used as a treatment for glaucoma, and to reverse atropine-induced coma during the last century. Recent uses for this compound and its derivatives have been as effective antidotes to several drugs which possess central anti-cholinergic properties.

During the last two decades, studies related to the acetylcholine-receptor-ion-channel complex (AChR)

of the neuromuscular junction have provided significant increases in knowledge of the receptor function. This membrane receptor has been readily available for study since nicotinic AChRs occur at very high densities in Torpedo and Electrophorus electric organs. Further, the understanding of the morphology and function of this receptor has been increased significantly by specific chemical probes for the different active sites of the receptor.

Nearly 20 years ago a significant discovery was made which helped in the study of this AChR. Alpha-bungarotoxin (Alpha-PGT) was obtained from snake venoms which binds irreversibly and specifically to the acetylcholine (ACh) recognition site on the nicotinic AChR. Alpha-PGT was such a highly selective probe that researchers were able to isolate and purify the different sub-units which comprise the nicotinic AChR. The sub-units were functionally reconstituted into artificial lipid membranes and were ultimately cloned.

Further sites on the nicotinic AChR were soon made available by the discovery of another class of toxins. These toxins were called histrionicotoxins and were isolated from the skin secretion of frogs in the family Dendrobatidae. The new sites available because of the histrionicotoxins were discovered to be responsible for the allosteric alterations or non-competitive blockage of neuromuscular transmission. These sites are distinct from the against recognition site discovered through the alpha-PGT probe and are thought to be located on the ion channel component of the AChR.

Further, other drugs demonstrate the ability to modify non-competitively the activation of the AChR. Examples of such drugs are distinct and well known

pharmacological agents which act on the peripheral nervous system as well as in the central nervous system. In particular, tricyclic anti-depressants, phenothiazine antipsychotics, the hallucinogenic agent 5 Phencyclidine (PCP), local anesthetics, antimuscarinics, anticholinesterase agents and similar compounds to mention but a few.

Further ways for studying AChR are available due to microscopic kinetic models and biochemical rapid 10 mixing methods to study permeability changes initiated by the binding of agonist molecules and conformational transitions of nicotinic receptor molecules.

The agonist recognition site at the nicotinic ACh receptor has been reported as having strong stereospecificity. This conclusion is based on the study of 15 optical isomers of certain semi-rigid agonists, see for example Spivak et al., Mol. Pharmacol., Vol. 23, pages 337-343 (1983).

Conversely, the ion channel sites are apparently 20 not stereospecific. This conclusion is based on the similar quantitative and qualitative actions of enantiomers of perhydrohistrionicotoxin at the nicotinic AChR, see for example Spivak et al., FEBS Lett. Vol. 163, pages 189-193 (1983).

It has been discovered that the natural isomer of 25 physostigmine has blocking properties as well as agonist properties at the neuromuscular AChR. By contrast (+)-physostigmine shows only negligible inhibition of cholinesterase (ChE). See Brossi et al., FEBS Lett., Vol. 201, pages 190-192 (1986).

Even though (+)-physostigmine has only negligible ChE inhibitory activity it is every effective as a protective pretreatment drug against multiple lethal

5 doses of sarin, see Albuquerque et al, Fundam. Appl. Caltoxicol., Vol. 5, pages 182-203 (1985). The observed beneficial protection appears to be due to direct interactions of the carbamates with the postsynaptic nicotinic AChR. The protective effectiveness of the carbamates against organophosphates appears to be related to the direct ability of the carbamates to decrease the hyperactivation caused by accumulation of the neurotransmitter.

10 The above information, available due to the research in this field, is important in the evaluation of potential new pharmacological agents for treating cholinergic disorders, for example, Myasthenia Gravis and Alzheimer's disease. Potential agents can be evaluated for potency in vitro by testing the agents 15 against electric eel acetylcholinesterase (AChE) and human plasma butyrylcholinesterase (BChE).

20 Of the two enzymes known to hydrolyze acetylcholine (ACh) in vivo, AChE, which is found in red blood cells, in the brain and in nerve tissues, seems to be more specific than BChE which is found in serum, pancreas and in the liver. It, however, has not previously been shown in the art that compounds 25 which selectively inhibit one of the two enzymes more than the other would offer a medical advantage. The natural alkaloid (-)-physostigmine, its potential metabolite (-)-(N1)-norphysostigmine, and the natural alkaloid physovenine which are used as biological standards in this art area inhibit AChE and BChE in 30 vitro similarly at similar concentrations.

Accordingly, there is need in the art for highly selective agents active against one of AChE and BChE and not very potent against the other which may lead to

better treatment of a particular cholinergic disorder and minimize negative side effects. Such compounds would be of great medical importance in the treatment of cholinergic disorders.

Summary of the Invention

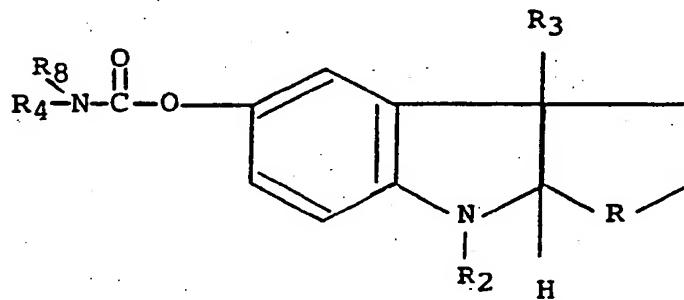
It is an object of the present invention to overcome the difficulties in the prior art as set forth in the background of the invention.

It is another object of the present invention to provide highly potent and selective cholinergic agonist and blocking compounds.

It is a further object of the present invention to provide improvements in therapy relative to cholinergic diseases such as glaucoma, Myasthenia Gravis, Alzheimer's disease, and organophosphate poisoning.

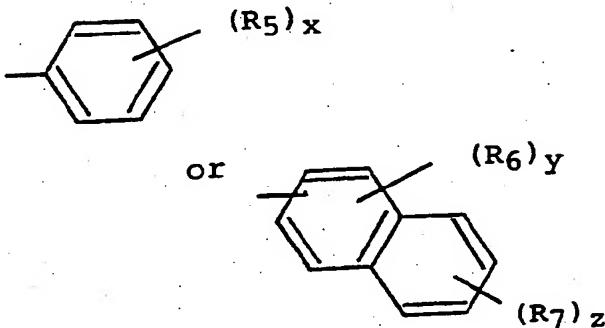
It is a still further object of the present invention to provide compounds with selective acetylcholinesterase and butyrylcholinesterase activity.

It is a yet further object of the present invention to provide compounds having the following formula:



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wherein R is -O- or the group -N(-R₁)- and
 R₁ is H or a -C₁-C₁₀-alkyl group;
 R₂ and R₃ are independently selected from H or
 -C₁-C₁₀-alkyl;
 R₄ is



wherein

R₅, R₆ and R₇ are independently selected from H, halogen or -C₁-C₁₀-alkyl;

x is 0 or an integer from 1-5,

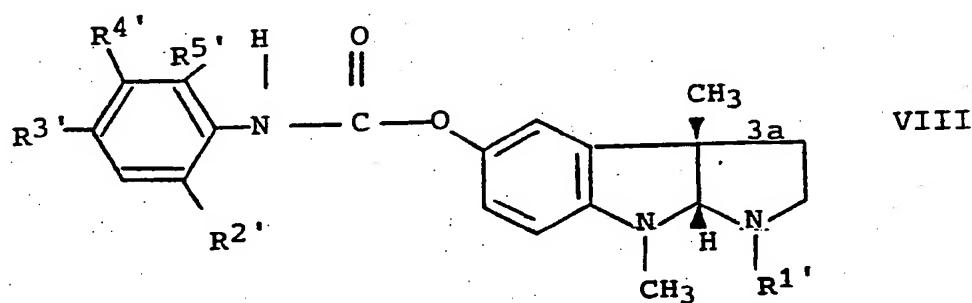
y is 0 or an integer from 1-3, and

z is 0 or an integer from 1-4; and

R₈ is H or C₁-C₁₀-alkyl;

including isomeric forms and pharmaceutically acceptable salts.

It is a still further object of the present invention to provide compounds having the following formula VIII :



wherein R^{1'} is H, a -CH₃ group or a benzyl group; R^{2'} is H or straight or branched chained C₁-C₁₀ alkyl; R^{3'} is H or straight or branched chained C₁-C₁₀-alkyl; and

R^{4'} and R^{5'} are independently hydrogen or R^{4'} and R^{5'} taken together along with the carbon atoms to which they are attached form a 6-membered aromatic hydrocarbon ring;

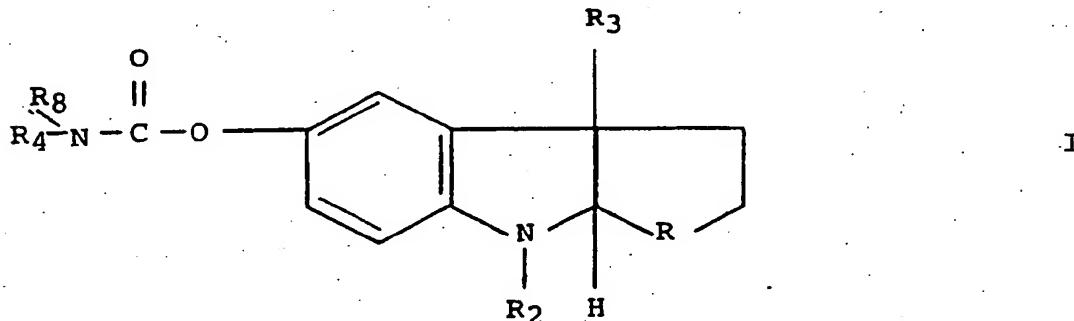
including isomeric forms and pharmaceutically acceptable salts.

Brief Description of the Figure

Figure 1 illustrates the time-dependent inhibition of plasma AChE in a rat host by physostigmine and its 2',4'-dimethylphenyl carbamate.

Description of Preferred Embodiments

In accordance with this invention there are disclosed compounds of the formula



wherein R is -O- or the group -N(-R₁)- and

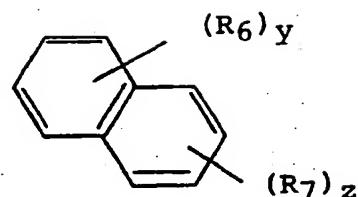
R₁ is H or a C₁-C₁₀-alkyl group;

R₂ and R₃ are independently selected from H or C₁-C₁₀-alkyl;

R₄ is



or



wherein

R₅, R₆ and R₇ are independently selected from H, halogen or -C₁-C₁₀-alkyl,

x is 0 or an integer from 1-5,

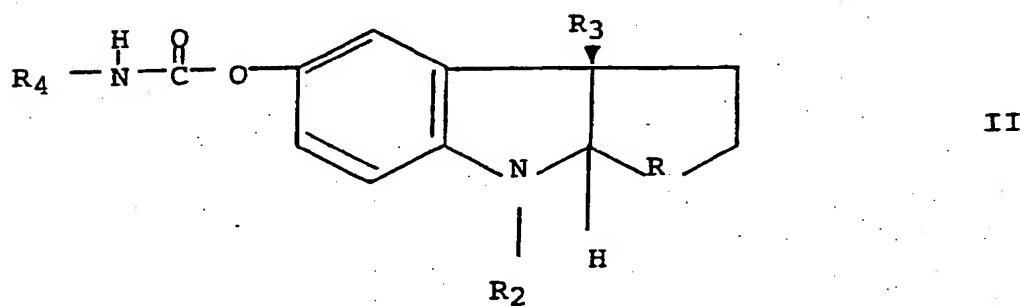
y is 0 or an integer from 1-3, and

z is 0 or an integer from 1-4, and

R₈ is H or -C₁-C₁₀-alkyl;

including isomeric forms and pharmaceutically acceptable salts.

Preferred are compounds according to Formula I having the Formula II:

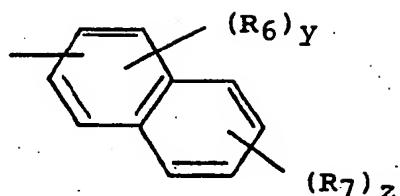


wherein R is -O- or the group -N(-R₁)- and
R₁ is H or a -C₁-C₁₀-alkyl group;

R_2 and R_3 are independently selected from H or
 $-C_1-C_{10}$ -alkyl; and
 R_4 is



or



wherein

R_5 , R_6 , and R_7 are independently selected from H, halogen or $-C_1-C_{10}$ -alkyl,

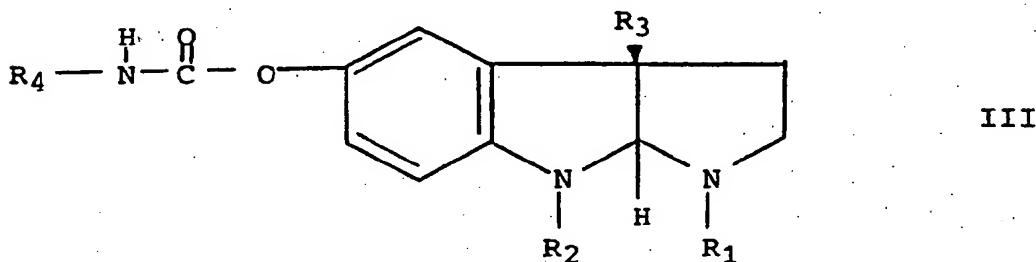
x is 0 or an integer from 1-5,

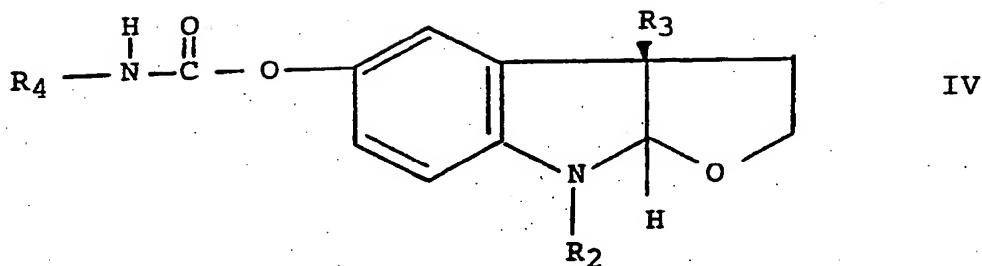
y is 0 or an integer from 1-3, and

z is 0 or an integer from 1-4;

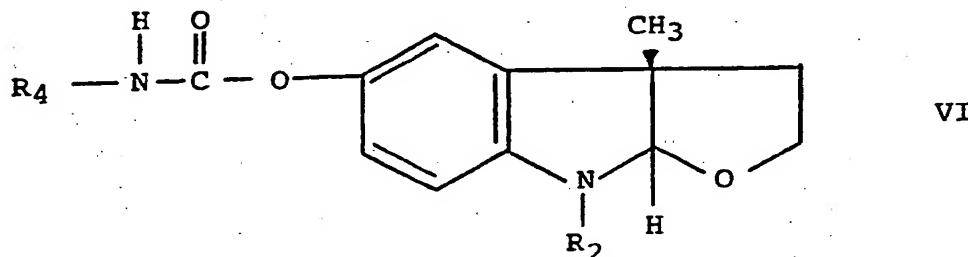
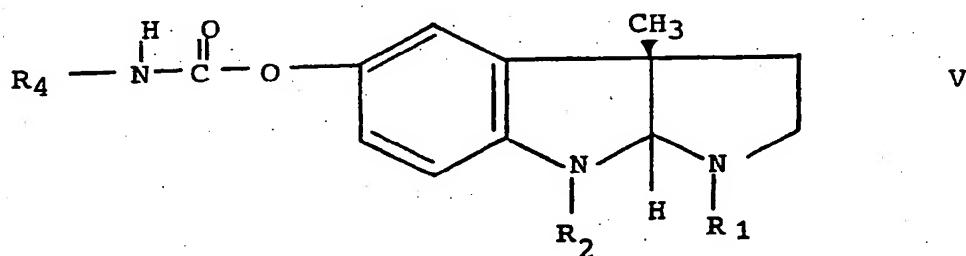
including isomeric forms and pharmaceutically acceptable salts.

Further preferred are compounds according to Formula II having the following Formula III and IV:

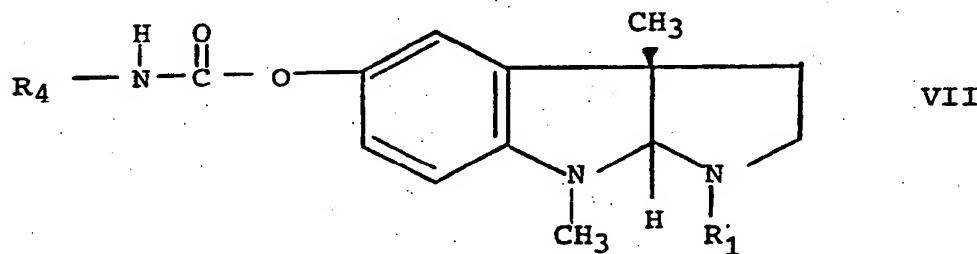




Still further preferred are compounds according to Formulas III and IV having the following Formula V and VI:



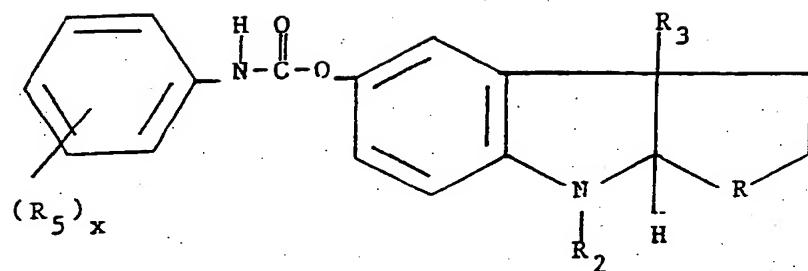
Yet further preferred are compounds according to Formula V having the following Formula VII



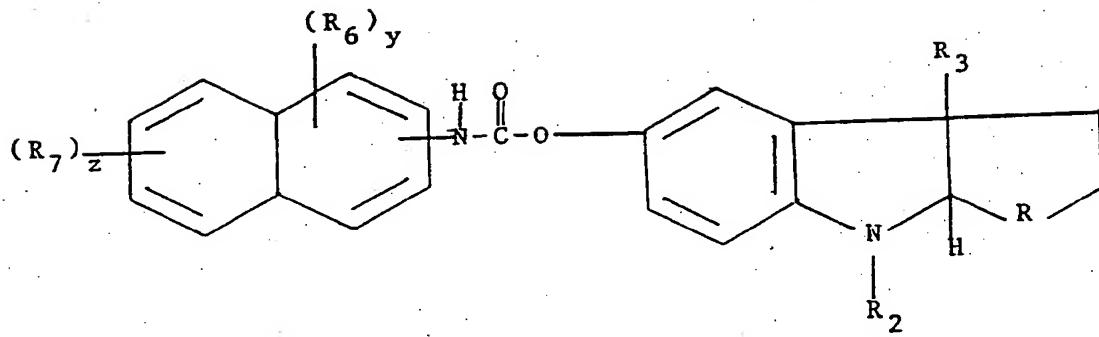
R_1-R_7 structures (where present) in the above Formulae III-VII are the same substituents defined above for Formula II.

Still further preferred are compounds of Formulae I-VII wherein x, y and z are 1 or 2. Even more preferred are compounds wherein x is 1 or 2 and R_5 is in the ortho and/or paraposition on the benzene ring. Particularly preferred R_5 groups are H, halogen and C_1-C_5 alkyl. Even more preferred R_5 groups are H, chloro, $-CH_3$, $-CH_2-CH_3$, $-CH_2-CH_2-CH_3$ and $-CH(-CH_3)_2$.

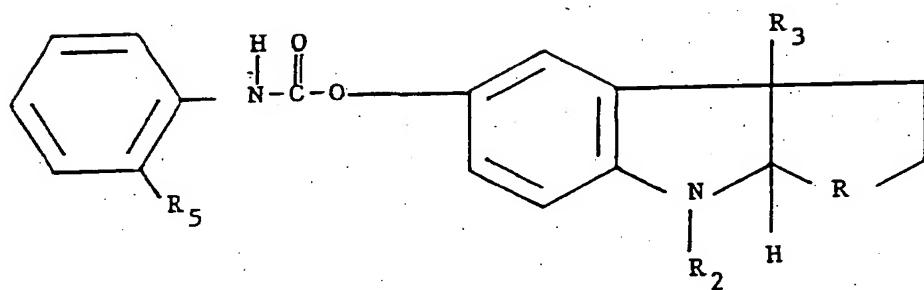
Preferred structures are set forth below wherein the main formula Roman numeral is further indicated with a lower case a, b, c or d in order to describe preferred groups for the R_4 substituent on each of the main formula which the Roman numerals alone represent, e.g., Ia-IId, IIa-IIId, etc.:



Ia

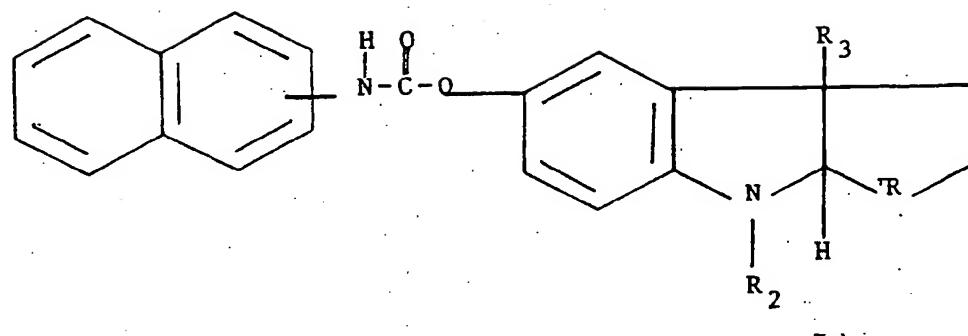


Ib

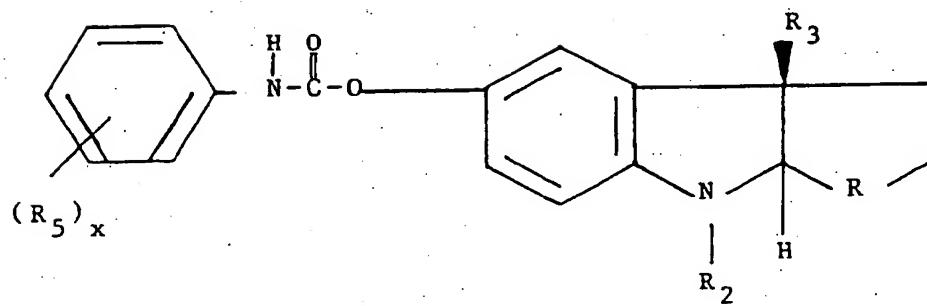


Ic

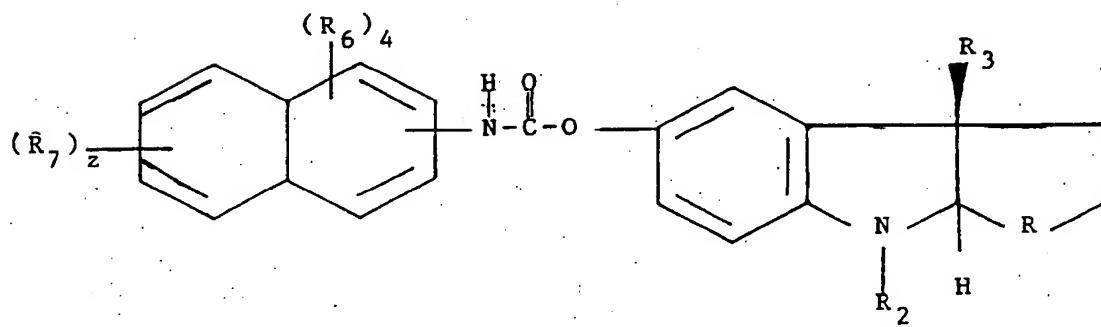
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Id

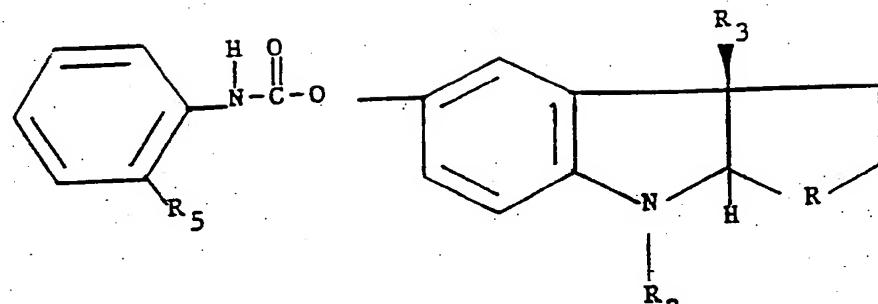


IIa

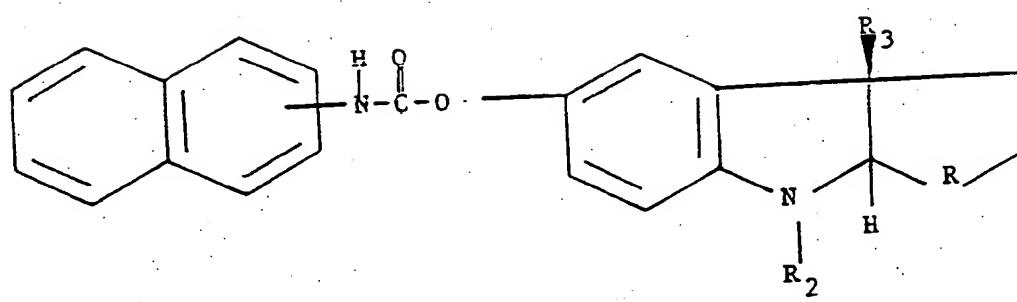


IIb

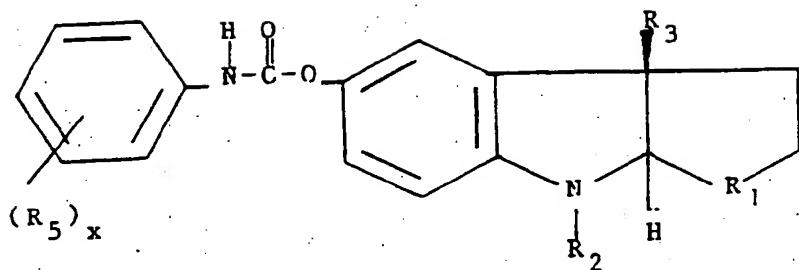
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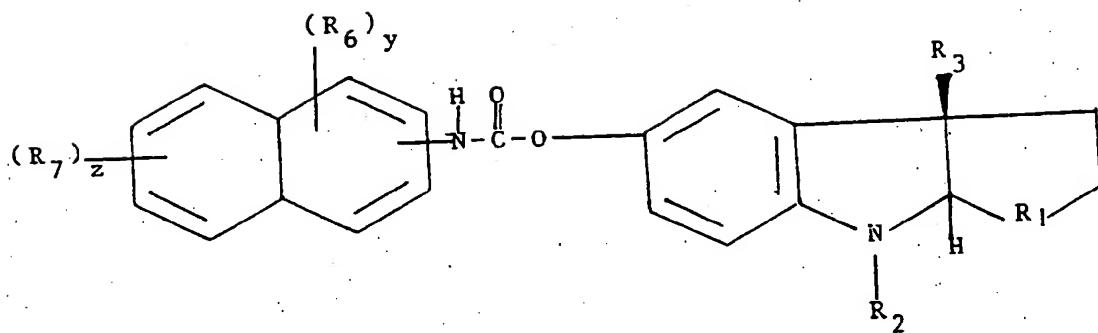
IIIc



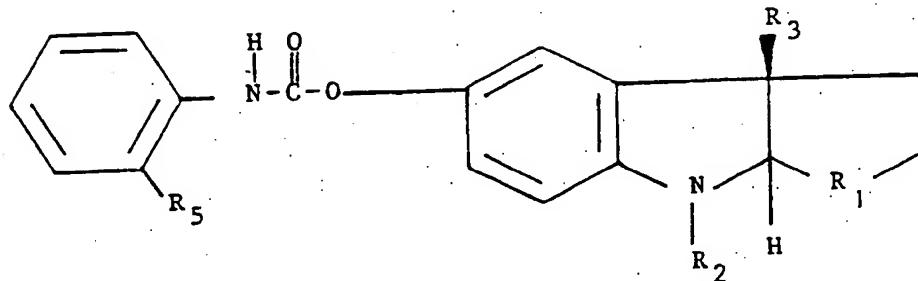
IIId



IIIa

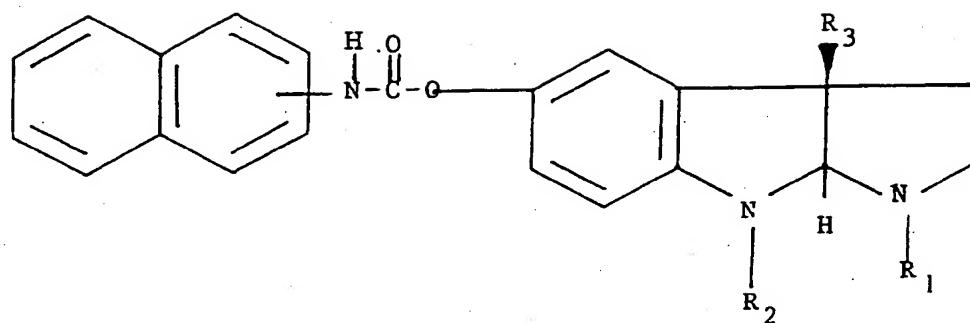


IIIb

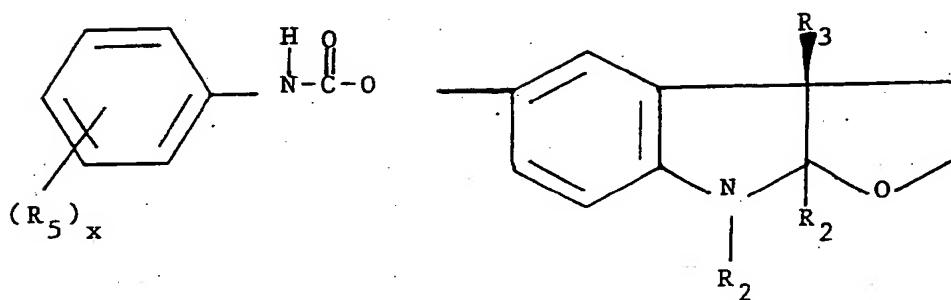


IIIc

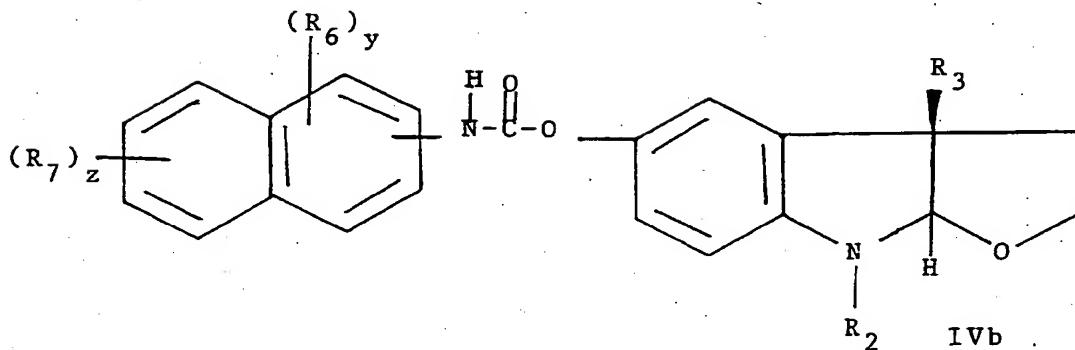
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III d

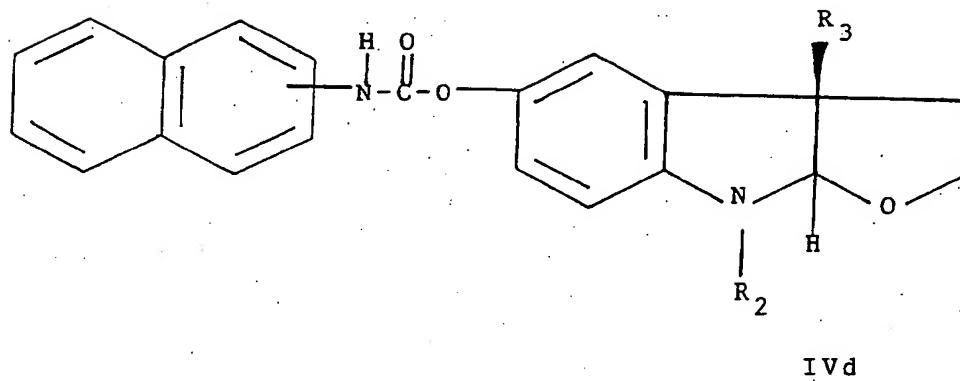
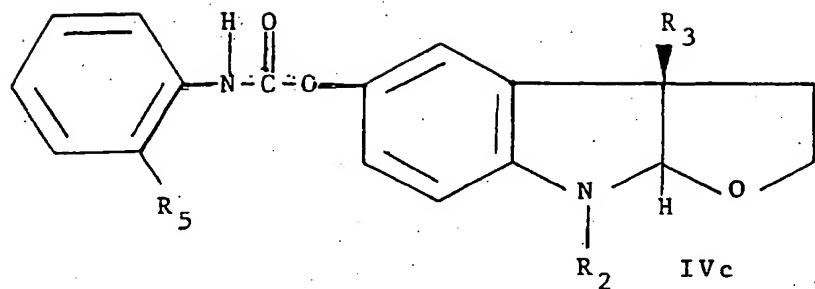


IV a

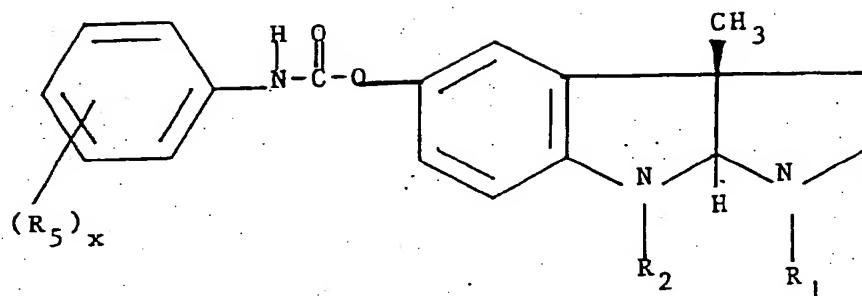


IV b

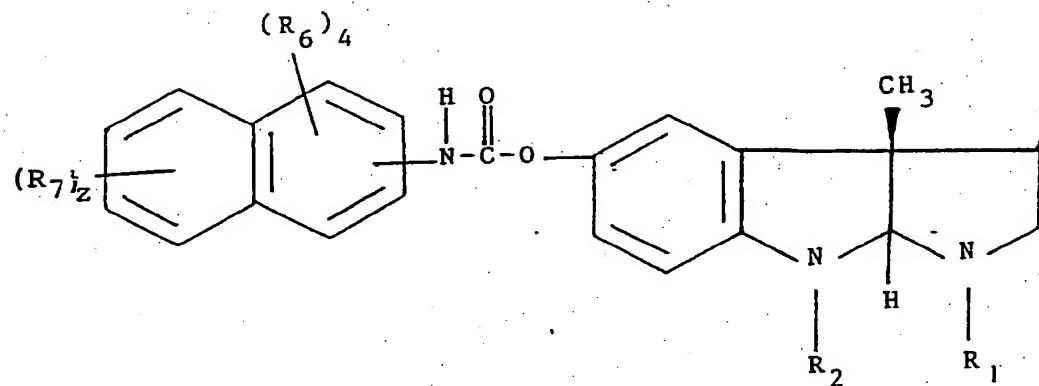
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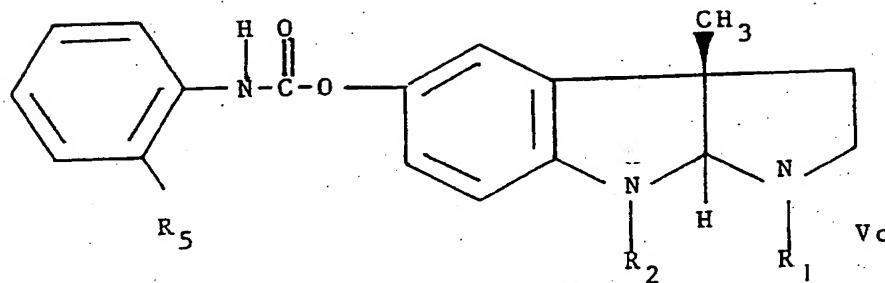
IVd



V a

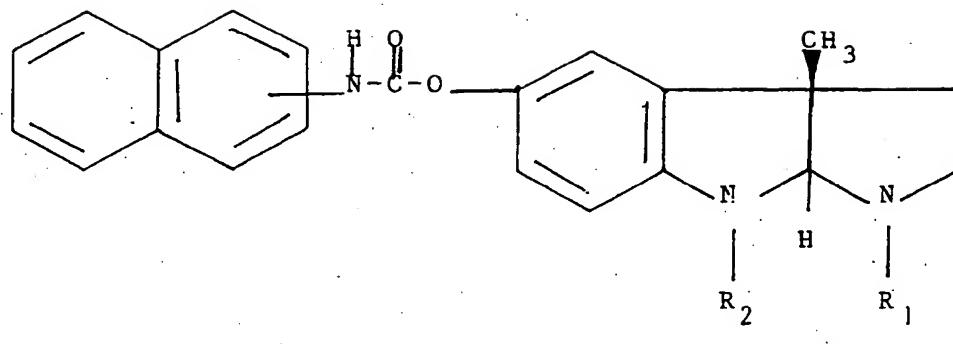


V b

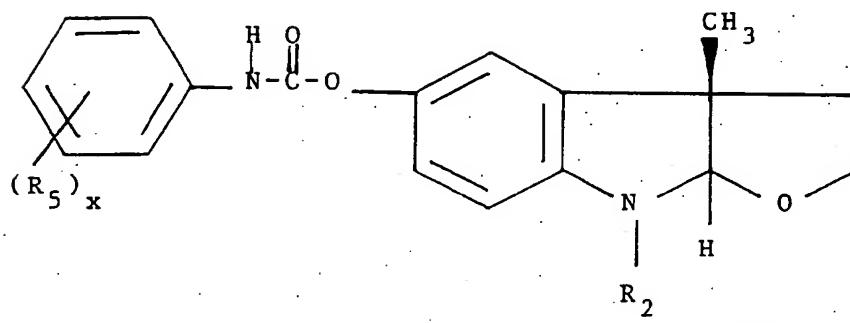


V c

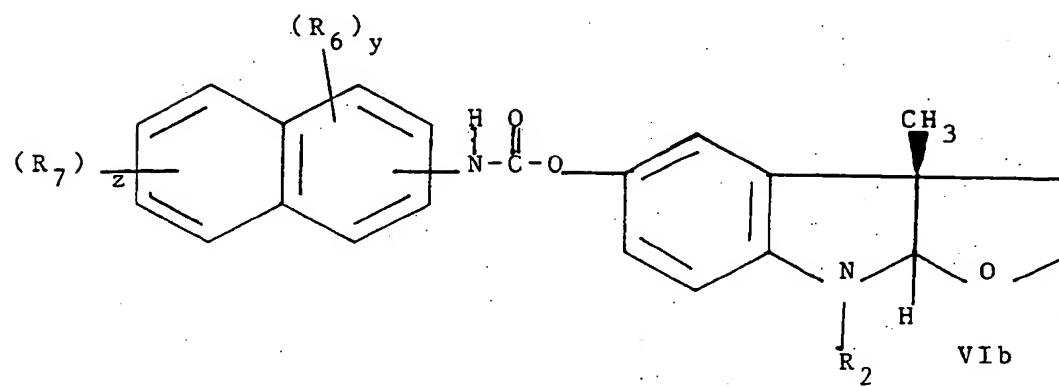
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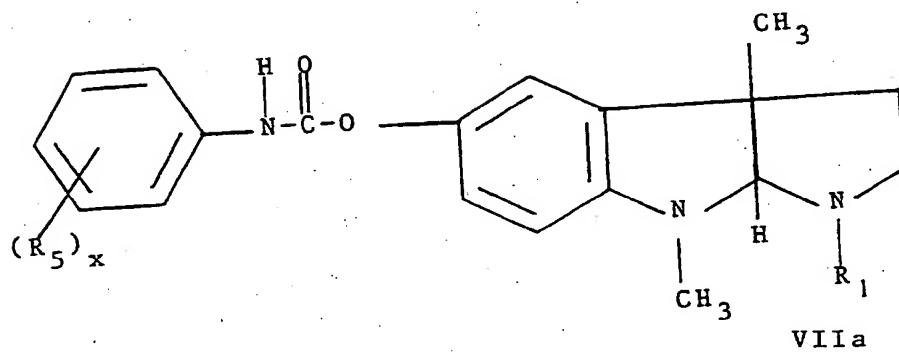
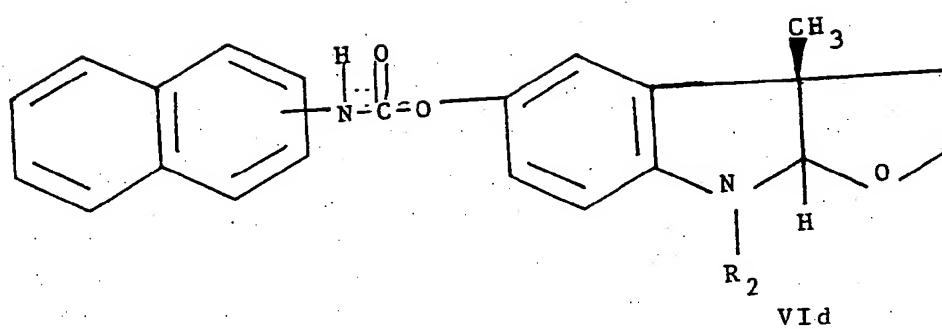
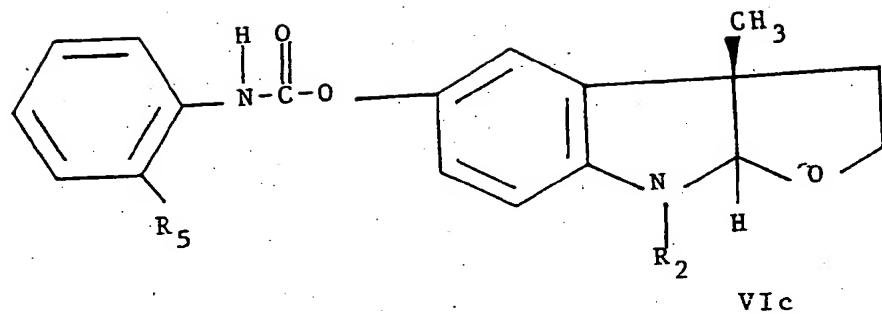
Vd



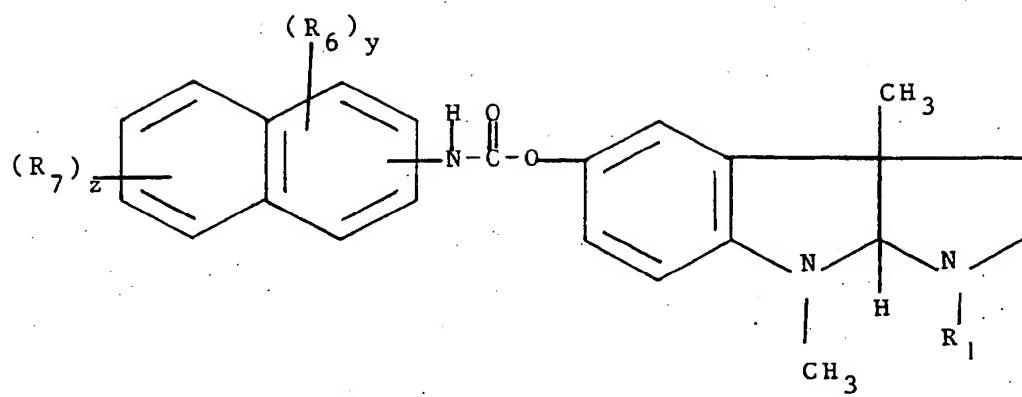
VIa



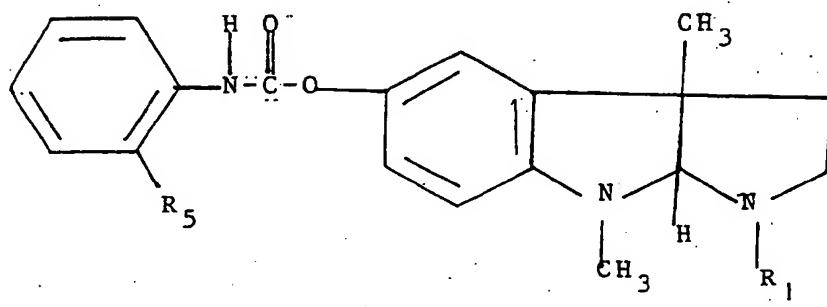
VIIb



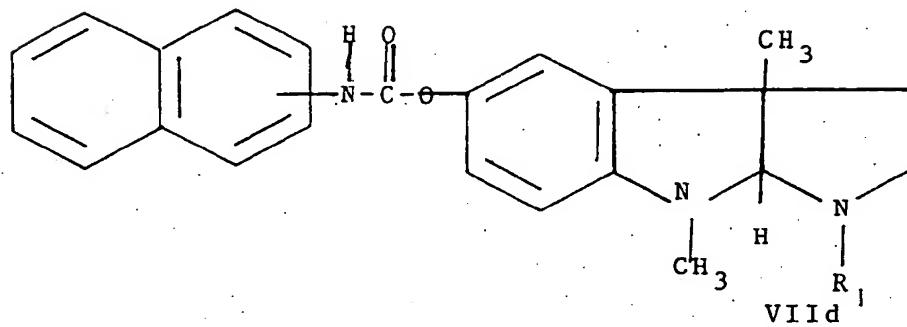
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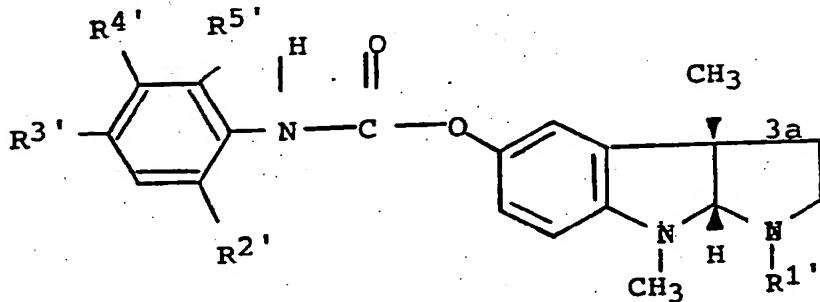
VIIb



VIIc



Also, in accordance with this invention there are disclosed compounds of the formula VIII



wherein R^1' is H, a $-CH_3$ group or a benzyl group;

R^2' is straight or branched chained C_1-C_{10} -alkyl;

R^3' is H or straight or branched chained C_1-C_{10} -alkyl; and

R^4' and R^5' are independently hydrogen or R^4 and R^5 taken together along with the carbon atoms to which they are attached form a 6-membered aromatic carbocyclic ring;

including isomeric forms and pharmaceutically acceptable salts. Acceptable salts are salts such as tartrates, fumarates, phosphates, salicylates, and the like.

Preferred are compounds of Formula VIII, wherein R^4' and R^5' are both hydrogen.

Even more preferred are compounds wherein R^3' is hydrogen and R^2' is C_1-C_{10} -alkyl. Yet more preferred is where these two groups are independently $-CH_3$, $-CH_2-CH_3$, $-CH_2-CH_2-CH_3$ and $-CH(-CH_3)_2$.

The above Formula VIII compounds are eseroline and (1) N-noreseroline carbamates having high potency in the inhibition of acetylcholinesterase and butyryl-cholinesterase. Some of the carbamates were more specific for AChE, whereas others were more highly specific for BChE.

Also preferred are compounds according to the present invention in isomeric forms and pharmaceutically acceptable salts thereof. Pharmaceutically acceptable salts can be, for example, the alkali metal, alkali earth and ammonium salt. Further, pharmaceutically acceptable organic and inorganic acid addition salts may be used. Other examples of acceptable salts are tartrates, fumarates, phosphates, salicylates, and the like.

The compounds according to Formula I have asymmetric carbon atoms and can exist as optical isomers. For the purpose of this invention, the racemic mixtures and dextro and laevo forms are included within the present invention. Hence, the particular dextro and laevo rotatory form or a particular isomer is sometimes indicated as a preferred optical isomer in particular formulae according to the invention.

Other cholinesterase inhibitors are known in the prior art. Physostigmine and physovenine are optically active alkaloids with a (3aS)-absolute configuration at the chiral carbon atom C(3a). Both of these compounds are potent inhibitors of cholinesterases in vitro and in vivo, blocking the conversion of acetylcholine into choline reversibly. Physostigmine has been found to have useful medical applications in disorders which result in a malfunction of this process.

Surprisingly, the carbamates according to the present invention have shown high potency. Thus, phenylcarbamate derivatives according to the present invention are longer lasting and appear to be less toxic than other carbamate analoges in this art.

Accordingly, the present compounds represent a significant advancement in the prior art.

Further, the above compounds according to the invention are useful as highly potent and selective cholinergic agonist and blocking pharmaceutical agents. Hence, the compounds of the present invention are useful in pharmaceutical compositions for systemic administration to human and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or suspensions, oral solutions or suspensions, oil and water or water in oil emulsions and the like, containing suitable quantities of the active ingredient. Topical application can be in the form of ointments, creams, lotions, jellies, sprays, douches, and the like. For oral administration either solid or fluid unit dosage forms can be prepared with the compounds of Formula I.

Compositions within the scope of the invention include compositions wherein the active ingredient is contained in an effective amount to achieve its intended purpose. The compounds can be administered in any pharmaceutically acceptable amount, for example, in amounts ranging from 0.001 gram to about 1 gram per kilogram of body weight. Based on the information which is presented herein, determination of effective amounts is well within the skill of the ordinary practitioner in the art. The compounds are generally useful in pharmaceutical compositions (wt%) of the active ingredient with a carrier or vehicle in the composition in about 0.1 to 99 wt% and preferably about 25-85 wt%.

Either fluid or solid unit dosage forms can be readily prepared for oral administration. For example, the compounds of Formula I can be admixed with conventional ingredients such as dicalcium phosphate, magnesium aluminum silicate, magnesium stearate, calcium sulfate, starch, talc, lactose, acacia, methyl cellulose and functionally similar materials as pharmaceutical excipients or carriers. The compounds according to the invention can also be administered as water soluble salts such as salicylates, oxalates, and such like. A sustained release formulation may optionally be used. Capsules may be formulated by mixing the compound with a pharmaceutical diluent which is inert and inserting this mixture into a hard gelatin capsule having the appropriate size. If soft capsules are desired a slurry of the compound with an acceptable vegetable, light petroleum or other inert oil can be encapsulated by making into a gelatin capsule.

Suspensions, syrups and elixirs may be used for oral administration of fluid unit dosage forms. A fluid preparation including oil may be used for oil soluble forms. A vegetable oil such as corn oil, peanut oil or safflower oil, for example, together with flavoring agents, sweeteners and any preservatives produces an acceptable fluid preparation. A surfactant may be added to water to form a syrup for fluid unit dosages. Hydro-alcoholic pharmaceutical preparations may be used having an acceptable sweetener, such as sugar, saccharin or a biological sweetener and a flavoring agent in the form of an elixir.

Pharmaceutical compositions for parenteral and suppository administration can also be obtained using techniques standard in the art.

A preferred use of the compounds according to the invention is as pharmaceutical agents suitable for oral administration. Another preferred use of the compounds is in transdermal parenteral cholinergic agonist and blocking pharmaceutical preparations, which are particularly useful in treating cholinergic disorders such as glaucoma, Myasthenia Gravis, Alzheimer's disease, and organophosphate poisoning. Accordingly, compositions suitable for administration to these areas are particularly included within the invention. The above parenteral solutions or suspensions may be administered transdermally and, if desired, a more concentrated slow release form may be administered. The above parenteral solutions or suspensions may be delivered with a skin patch. If desired these solutions or suspensions may be given by injection in an appropriate vehicle such as sesame oil.

Accordingly, incorporation of the active compounds and a slow release matrix may be implemented for administering transdermally. The compounds may be administered transdermally at about .01 to 99% of the composition and preferably about 25 to 85 wt% of the active ingredient in the vehicle or carrier.

Transdermal therapeutic systems are self-contained dosage forms that, when applied to intact skin, deliver drug(s) at a controlled rate to the systemic circulation. Advantages of using the transdermal routing include: enhanced therapeutic efficacy, reduction in the frequency of dosing, reduction of side effects due to optimization of blood-concentration vs.

time profile, increased patient compliance due to elimination of multiple dosing schedules, bypassing the hepatic "first pass" metabolism, avoiding gastrointestinal incompatibilities and providing a predictable and extendable duration of activity. However, the main function of the skin is to act as a barrier to entering compounds. As a consequence, transdermal therapy has been preferred for a limited number of drugs that possess the desirable physiochemical properties for diffusion across the skin barrier. One effective method of overcoming the barrier function of the skin is to include a penetration enhancer in the formulation of the transdermal therapeutic system.

The penetration enhancer is a chemical compound that, when included in a formulation, temporarily increases the permeability of the skin to a drug line allowing more of the drug to be absorbed in a shorter period of time. Several different types of penetration enhancers have been reported such as dimethylsulfoxide, n-decylmethylsulfoxide, N,N-dimethylacetamide N,N-dimethylformamide, 1-dodecylazacycloheptane-2-one (Azone), propylene glycol, ethanol, pyrrolidones such as N-methyl-2-pyrrolidone (NMP) and surfactants.

The above compounds can be present in the reservoir alone or in combination with pharmaceutical carriers. The pharmaceutical carriers acceptable for the purposes of this invention are the art known carriers that do not adversely effect the drug, the host, or the material comprising the drug delivery device. Suitable pharmaceutical carriers include sterile water; saline; dextrose; dextrose in water or saline; condensation products of castor oil and

ethylene oxide combining about 30 to 35 moles of ethylene oxide per mole of castor oil; liquid acid; lower alkanols; oils such as corn oil; peanut oil; sesame oil and the like, with emulsifiers such as mono- or di-glyceride of a fatty acid; or a phosphatide, e.g., lecithin; and the like; glycols; polyalkylene glycols; aqueous media in the presence of a suspending agent, for example, sodium carboxymethyl cellulose; sodium alginate; poly(vinylpyrrolidone); and the like, alone, or with suitable dispensing agents such as lecithin; polyoxyethylene stearate; and the like. The carrier may also contain adjuvants such as preserving, stabilizing, wetting, emulsifying agents and the like together with the penetration enhancer and the compounds of this invention.

The effective dose for mammals may vary due to such factors as age, weight, activity level or condition of the subject being treated. Typically, an effective dosage of a compound according to the present invention is about 1 to 800 milligrams when administered by either oral or rectal dose from 1 to 3 times daily. This is about .002 to about 50 milligrams per kilogram of the subject's weight administered per day. Preferably about 10 to about 300 milligrams are administered orally or rectally 1 to 3 times a day for an adult human. The required dose is considerably less when administered parenterally, preferably about .01 to about 150 milligrams may be administered intramuscularly or transdermally, one or two times a day for an adult human.

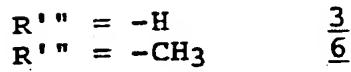
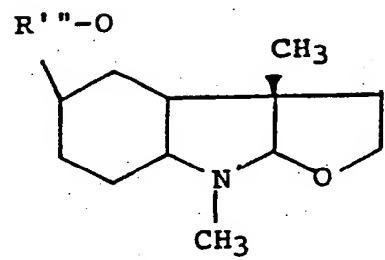
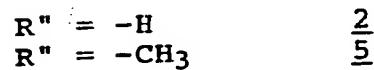
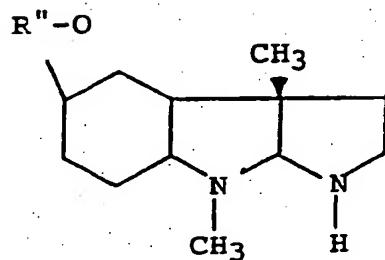
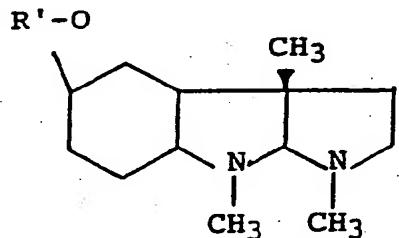
Compounds of the present invention may be administered topically at about .01 to about 99 wt% of the composition, and preferably about 25 to 85 wt%.

The present compounds are also useful in a method for treating cholinergic disorders such as glaucoma, Myasthenia Gravis, Alzheimer's disease, and as an antidote against poisoning with organo phosphates. The method according to the invention comprises some interesting effective amount of a compound according to the invention or an effective amount of a pharmaceutical composition according to the invention to a mammal in need of such treatment.

Surprisingly, the compounds according to the invention have shown selective cholinergic agonist and blocking activity. Of the two enzymes known to hydrolyze acetylcholine *in vivo*, acetylcholinesterase (AChE) which is found in red blood cells, in the brain, and in nerve tissues, seems to be more specific than butyrylcholinesterase (BChE) which is found in serum, pancreas and in the liver. It, however, was never shown that compounds which selectively inhibit one of the two enzymes more than the other, would offer a medical advantage.

The present invention relates to selective inhibition as follows. The natural alkaloid (-)-physostigmine, its potential metabolite (-)-(N1)-norphysostigmine and the natural alkaloid physovenine which were used as biological standards in the inhibited AChE and BChE *in vitro* similarly at similar concentrations.

These biological standard compounds used for comparative purposes and derivatives having protective groups have the following structures.



The above structures are also used as starting materials to produce compounds according to the present invention.

The phenylcarbamate of (-)-eseroline and referred to in the literature as phenserine, however, was determined by the present invention experimentation to inhibit AChE from human erythrocytes in vitro at a 50-times lower concentration than BChE from human plasma. Accordingly, further derivatives were made and tested.

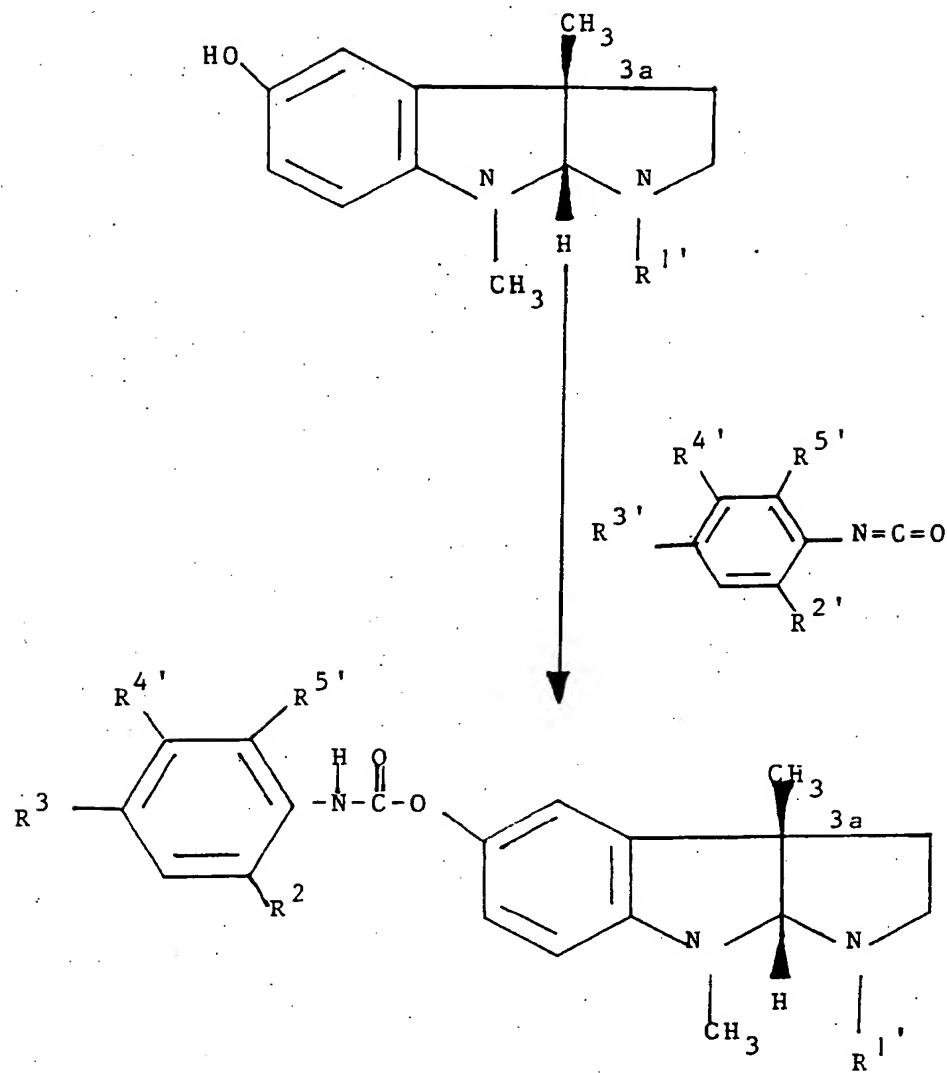
It was discovered according to the present invention that substituting the phenyl group in para-position with a methyl group, a chlorine atom, or a methoxy group afforded derivatives which inhibited both enzymes at similar concentrations but such derivatives were considerably less potent than the biological standards described above. The phenylcarbamate of (-)-physovenol (22), also showed high preference for AChE (IC_{50} for AChE = 11, and for RChE = 700), whereas the cumylcarbamate (4'-isopropylphenylcarbamate) (24) showed a reverse enzyme specificity (AChE = 3800 and for BChE = 16.5).

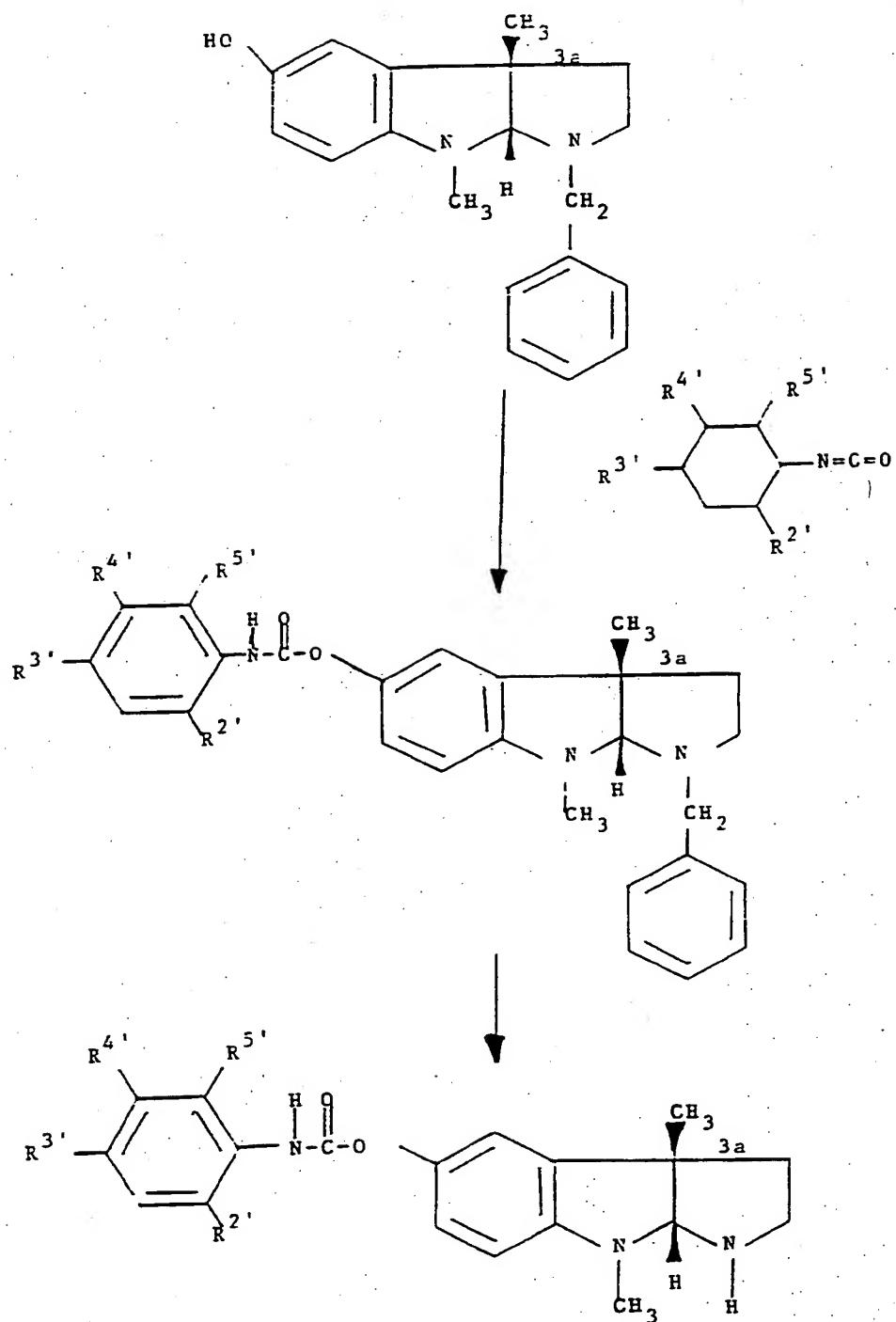
These above discoveries clearly indicated that selective inhibition of either AChE or BChE could be achieved by replacing the hydrogens on the phenyl group in phenylcarbamates with various substituents and inserting these modified phenylcarbamates on the basic core structure present in the three alkaloids that are the biological standards described above. The increased possibility of designing specifically acting inhibitors of AChE or BChE prompted an extension of these investigations and the results are the subject of the present invention.

The phenylcarbamates listed below in Table 1 and Table 2 were prepared from (-)-eseroline (4), (-)-(N1)-benzylnoreseroline (5) as the N-protected equivalent of (2), and from (-)-physovenol (6) which all have the natural (3aS)-absolute configuration (these numbers for the starting materials correspond to the numbers on the comparative structures and protected derivatives, whose structures are listed just previously in the above specification).

Reaction of phenols having the natural (3aS)-absolute configuration, i.e., (-)-eseroline, (-)-(N1)-noreseroline, or (-)-(N1)-benzylnoreseroline with commercially available isocyanates in dry ether and in the presence of a catalytic amount of sodium, afforded the desired carbamates. See Reaction Scheme 1, below. They were separated from "dimers", which invariably formed, by chromatography, and removed as the faster running materials. The structures of the carbamates which often were amorphous were secured by MS and ¹H-NMR spectra, and they were characterized by TLC-analysis and by optical rotation. Details of the preparation of the carbamates according to the present invention are given in the experimental section. Conversion of the (N1)-protected carbamates into compounds of the (N1)-series was accomplished by catalytic hydrogenation over Pd(OH)₂ catalyst as shown in Scheme 2 and described below.

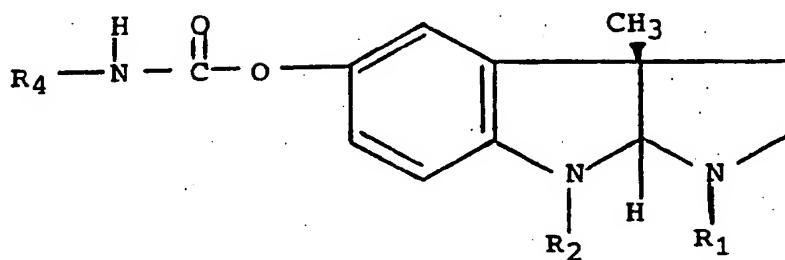
The resulting phenylcarbamates are listed below in Tables 1-3, following the illustration of reaction Schemes 1 and 2. The phenyl carbamates of Formula VIII, which all have the natural (3aS)-absolute configuration, are listed below in Table 3.

SCHEME 1**SUBSTITUTE SHEET**

SCHEME I**SUBSTITUTE SHEET**

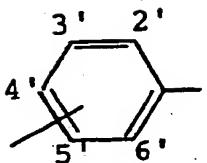
Compounds according to the present invention, i.e., compounds 7-24, are listed in Table 1 and Table 2, below.

Table 1



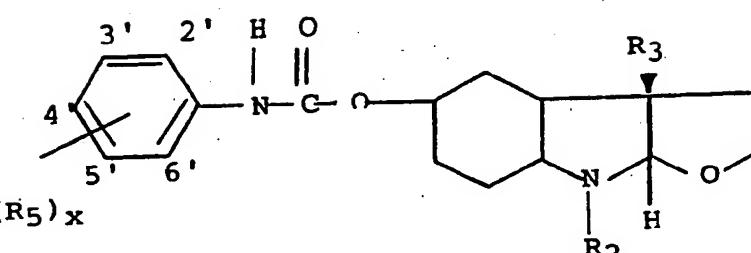
	<u>R₄</u>	<u>x</u>	<u>R₁</u>	<u>R₂</u>	<u>R₅</u>
<u>7</u>	 (R ₅) _x	1	-CH ₃	-CH ₃	2'-CH ₃
<u>8</u>	"	2	-CH ₃	-CH ₃	2', 4'-CH ₃
<u>9</u>	"	1	-CH ₃	-CH ₃	4'-CH(CH ₃) ₂
<u>10</u>	"	1	-CH ₃	-CH ₃	4'-CH ₃
<u>11</u>	"	2	-CH ₃	-CH ₃	2', 6'-CH ₂ -CH ₃
<u>12</u>	"	1	-CH ₃	-CH ₃	2'-CH ₂ -CH ₃
<u>13</u>	"	1	-CH ₃	-CH ₃	2'-CH(-CH ₃) ₂
<u>14</u>	"	1	-CH ₃	-CH ₃	H
<u>15</u>	"	3	-CH ₃	-CH ₃	2', 4', 6'-CH ₃
<u>16</u>		-	-CH ₃	-CH ₃	-

Table 1 (Continued)

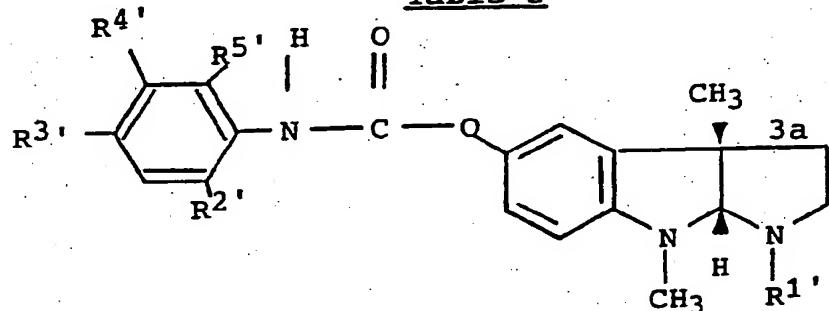
	<u>R₄</u>	<u>x</u>	<u>R₁</u>	<u>R₂</u>	<u>R₅</u>
<u>17</u>		1	-CH ₃	-CH ₃	2'-Cl
	(R ₅) _x				
<u>18</u>	"	2	-CH ₃	-CH ₃	2', 6'-Cl
<u>19</u>	"	1	-H	-CH ₃	2'-CH ₃
<u>20</u>	"	2	-H	-CH ₃	2', 4'-CH ₃
<u>21</u>	"	1	-H	-CH ₃	4'-CH(-CH ₃) ₂

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Table 2

R	X	R ₂	R ₃	R ₅
				
<u>22</u>	-O-	1	-CH ₃	-CH ₃
<u>23</u>	"	1	-CH ₃	-CH ₃
<u>24</u>	"	1	-CH ₃	2'-CH ₃
				4'-CH(-CH ₃) ₂

Compounds according to the present invention, i.e., compounds 25-38 and comparative compounds A', B', and C' are listed in Table 3, below.

Table 3

$R^{4'}, R^{5'}$ R^1' $R^{2'}$ $R^{3'}$

25.	-H, -H	-CH ₃	-CH ₃	-H
26.	-H, -H	-CH ₃	-CH ₃	-CH ₃
27.	-H, -H	-CH ₃	-H	-CH(-CH ₃) ₂
28.	-H, -H	-CH ₃	-CH ₂ -CH ₃	-H
29.	-H, -H	-CH ₃	-CH(CH ₃) ₂	-H
30.		-CH ₃	-H	-H
31.	-H, -H	-CH ₂ -Ph	-CH ₃	-H
32.	-H, -H	-CH ₂ -Ph	-CH ₃	-CH ₃
33.	-H, -H	-CH ₂ -Ph	-H	-CH(CH ₃) ₂
34.	-H, -H	-CH ₂ -Ph	-H	-H
35.	-H, -H	-H	-CH ₃	-H
36.	-H, -H	-H	-CH ₃	-CH ₃
37.	-H, -H	-H	-H	-CH(CH ₃) ₂
38.	-H, -H	-H	-H	-H
A'.	-H, -H	-CH ₃	-H	-CH ₃
B'.	-H, -CH ₂ CH ₃	-CH ₃	-CH-CH ₃	-H
C'.	-H, -CH ₃	-CH ₃	-CH ₃	-CH ₃

Experimental

Melting points (uncorrected): Fisher-Johns apparatus; optical rotations ($[\alpha]_D$, CHCl_3 : Perkin-Elmer-241 MC automatic polarimeter, IR spectra (cm^{-1} , CHCl_3): Beckman-IR-4230 instrument, BIO-RAD FTS-45 instrument; ^1H NMR (in CDCl_3 with Me_4Si as internal reference, δ ppm, J Hz): Varian XL-300 MHz, Gemini 300 MHz spectrometer, MS (m/z) for chemical ionization (CI): Finnigan-1015D mass spectrometer, for electron impact (EI): V.G. Micromass 7070 mass spectrometer, for HR MS (FAB): JEOL JMS-SX 102 magnetic sector mass spectrometer thin layer chromatography (silica gel GHLF), 250 μm): Analtech Inc.; column chromatography (silica gel GHLF, 250 μm): Merck 60 (230-400 mesh); the solvent systems used for TLC analysis were the following: $\text{CH}_2\text{Cl}_2/5\%$ MeOH; $\text{CH}_2\text{Cl}_2/10\%$ MeOH; the solvent systems used for column chromatography: $\text{CH}_2\text{Cl}_2/5\%$ MeOH(A); $\text{CH}_2\text{Cl}_2/10\%$ MeOH(B).

(-)-2'-Methylphenylcarbamoyleseroline (7):

(-)-Eseroline (4) 0 (0.12 g, 0.55 mmol) was dissolved in anhydrous Et_2O (10 mL) and a small piece of Na metal was added. After stirring for about 2 min at room temperature under nitrogen, 2-methylphenylisocyanate (0.09 g, 0.70 mmol) was added dropwise. After complete addition the solvent was evaporated immediately. The residue was flash chromatographed on a silica gel column (system B) to give (7) as a foam (0.88g, 46%); $[\alpha]_D -69.6^\circ$ ($c=0.5$, CHCl_3), CI MS (m/z): 352 ($\text{M}^{+}+1$); EI MS (m/z): 351 (M^{+}), HR MS (FAB) calcd for ($\text{M}^{+}+1$) $\text{C}_{21}\text{H}_{26}\text{N}_3\text{O}_2$ 352.2025, found 352.2020, IR; 3410, 2930, 1745; ^1H NMR 1.46 (s, 3H,

C₁₀-CH₃), 1.90-2.12 (m 2H, C₃-H), 2.32 (s, 3H, Me-Ph), 2.55 (s, 3H, N₁-CH₃), 2.58-2.70 (m, 2H, C₂-H₂), 2.91 (s, 3H, N*-CH₃), 4.18 (s, 1H, C₉-H), 6.33 (d, J = 8.4, 1H, C₇-H), 6.63 (br s, 1H, N-H), 6.85-6.95 (m, 2H, C₄-H, C₆-H), 7.05 (t, J = 1H, C_{5'}-H), 7.15-7.23 (m, CH, CH_{3'}-H, C_{6'}-H), 7.85 (br s, 1H, C_{4'}-H).

All other carbamates: (-)-2'-4'-dimethylphenylcarbamoyleseroline (8), (-)-4'-isopropylcarbamoyleseroline (9), (-)-4'-methylphenylcarbamoyleseroline (10), (-)-2',6'-diethylphenylcarbamoyleseroline (11), (-)-2'-ethylphenylcarbamoyleseroline (12), (-)-2'-isopropylphenylcarbamoyleseroline (13), (-)-phenylcarbamoyleseroline (14), (-)-(-)-2',4',6'-trimethylphenylcarbamoyleseroline (15), naphthylcarbamoyleseroline (16), (-)-2'-chlorophenylcarbamoyleseroline (17) and (-)-2',6'-dichlorophenylcarbamoyleseroline (18) were similarly prepared from (-)-eseroline (4) with the corresponding isocyanates and showed similar IR and NMR spectra to (7). The important data for these compounds is shown in Table 4 below.

The carbamates: (-)-2'methylphenylcarbamoyl-N₁-noreseroline (19), (-)-2',4'-dimethylphenylcarbamoyl-N₁-noreseroline (20) and (-)-4'-isopropylphenylcarbamoyl-N₁-noreseroline (21) were similarly prepared from (-)-(N₁)-benzylnoreseroline (5) instead of (4) by reacting (5) with the corresponding isocyanates. The benzyl protecting group was then removed to yield the noreseroline compound from the benzylnoreseroline compound. The important data for these compounds is shown in Table 4 below.

The following example shows the removal of a benzyl protecting group from (-)-2'-Methylphenylcarbamoyl-(N1)-benzylinoreseroline to yield compound (19).

(-)-2'-Methylphenylcarbamoyl-N1-Noreseroline (19):

(-) - 2' - Methylphenylcarbamoyl - (N1) - benzylinoreseroline (0.09g, 0.21 mmol) was dissolved in MeOH (10 mL), and palladium hydroxide on carbon (7 mg) was added. After hydrogenation under atmospheric pressure for 5 h, the palladium catalyst was filtered through Celite and the solvent was evaporated in vacuo. The residue was chromatographed by preparative TLC (silica gel plate 2000 μ m, CH_2Cl_2 /10% MeOH) to give compound (19) as a foam (0.04g, 56%): $[\alpha]_D^{25} -62.4^\circ$ ($c = 0.5$, CHCl_3), CI MS (m/z): 338 ($M^+ + 1$); EI MS (m/z): 337 (M^+), HR MS (EI) (M^+) calcd, for $C_{20}\text{H}_{23}\text{N}_3\text{O}_2$ 337.1790, found 337.1776 ^1H NMR: 1.42 (s, 3H, C10- CH_3), 1.70-1.80 (m, 1H, C3-H), 1.95-2.08 (m, 1H, C2-H), 2.29 (s, 3H, C2'- CH_3), 2.70-2.80 (m, 1H, C2-H), 2.81 (s, 1H, N8- CH_3), 3.01-3.10 (ddd, $J = 2.5, 2.5, 2.5$, 1H, C2-H), 4.51 (s, 1H, C9-H), 6.25 (d, $J = 9.0$, 1H, C7-H), 6.63 (br s, 1H, N-H), 6.83-6.86 (m, 2H, C4-H, C6-H), 7.02 (t, $J = 7.5$, 1H, C5'-H), 7.15-7.22 (m, 2H, C3'-H, C6'-H), 7.85 (br s, 1H, C4'-H).

Compounds (20) and (21) showed similar IR and NMR spectra to compound (19), see Table III below.

(-) - 5-O- (2' - Methylphenylcarbamoyl) physovenol (23):

(-) - Physovenol (6) (0.042 g. 0.20 mmol) was dissolved in anhydrous Et_2O (8 mL) and a small piece of Na metal was added. After stirring for about 2 min at room temperature under nitrogen, 2-methylphenyl-isocyanate (0.032 g, 0.24 mmol) was added dropwise. After complete addition the reaction mixture was

stirred at room temperature for an additional 1 h and then refluxed for 1.5 h. The solvent was evaporated and the residue was flash chromatographed on a silica gel column (system B) to give (23) as a foam (not TLC pure). This material was further purified by preparative HPLC on an Axiom silica column (5 μ , 10 x 250 mm) using 1.5% MeOH in CH₂Cl₂ at a flow rate of 5 mL/min. The product thus obtained (.03 g, 43%) as a foam was TLC pure: $[\alpha]_D^{25}$ -31.0° (c=1.0 CHCl₃), CI MS (m/z): 339 (M⁺+1); EI MS (m/z): 338 (M⁺); IR: 3400, 2950, 1740; ¹H NMR: 1.46 (s, 3H, C10-CH₃), 1.95-2.20 (m, 2H, C3-H), 2.32 (s, 3H, C2'-CH₃), 2.91 (s, 3H, N8-CH₃), 3.40-3.55 (ddd, J = 5.3; 8.6; 11.0, 1H, C2-H), 3.98 (dt, J = 1.4; 8.6 H, C2-H), 5.10 (s, 1H, C9-H), 6.31 (d, J = 9.0, 1H, C7-H), 6.55 (br s, 1H, N-H), 6.85 (m, 2H, C4-H, C6H), 7.05 (t, J = 7.4 1H, C5'-H), 7.13 (m, 2H, C3'-H, C6'-H), 7.86 (br s, 1H, C4'-H).

Compounds (22) and (24) were produced similarly to compound (23) by substituting phenylisocyanate and 4-isopropylphenylisocyanate, respectively, for the 2-methylisocyanate in the above procedure. Compounds (22) and (24) showed similar IR and NMR spectra to compound (23).

Table 4 below lists the important physical data for compounds according to the invention. The compound numbers in Table 4 correspond to the compound numbers in Table 1 and Table 2.

TABLE 4

$[\alpha]_D$ (°f) (c=1, CHCl ₃)	mp (°C)	CIMS (m/z) $m \pm 1$	HRMS m/z calcd	(FAB) (M ⁺ =1) (+)mmz	¹ H NMR
<u>8</u> -79.6	foam	366			2.28 (s, 3H, C2-CH ₃)
<u>9</u>					2.29 (s, 3H, C4' -CH-(CH ₃)
<u>10</u> -74.2	143-145	392	C ₂₁ H ₂₆ N ₃ O ₂	231 (s, 3H, C4' -CH ₃)	
<u>11</u> -36.1	oil	394	C ₂₄ -H ₃₂ N ₃ O ₂ 394, 2495(+0.3)	1.24 (d, J= 7.4, 6H, 2- CH ₂ -CH ₃) 2.68 (m, 5H)	
<u>12</u> -62.8	foam	366	C ₂₂ H ₁₈ N ₃ O ₂		1.26 (d, J=7.5, 3H, CH ₂ -CH ₃), 2.55- 2.78 (M, 4H, C2-H -CH ₂ -CH ₃)

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TABLE 4 Cont.14 -74.2

142-143 338

7.01 (d J =
7.4,
1H, C4' -
H), 7.22
(d, J =
7.4, 2H,
C3' -H, C5'
-H), 7.34
(d, J = 7.4,
2H, C2' -
H, (6' -H)

15 -55.8

foam

380

228 (3s,
CH, C2', C4',
C6-CH₃)

16 -62.0

foam

388 C₂₈C₂₆N₃O₂
3882025(-1.6)

7.51 (m, 3H),
7.69 (d, J =
8.1, 1H),
7.89 (d, J =
7.5, 1H),
7.96
(d, J = 7.9, 2H)

17 -67.2

oil

372

7.02 (dJ=7.8,
C4' -H)

18 -66.2

oil

406

7.19 (dJ=7.8,
C4' -H), 7.39
(d, J = 7.8,
C3', C5' -H)

15 -60.7

126-127

324(M⁺)C=0.6
(24) -54.6

167-169

397

1.24 (d, J=7.0,
2.90 (m
superimposed
with N-CH₃,
4H, CH-iPr)

EXAMPLE 25: (-)-2'-Methylphenylcarbamoyleseroline

(-)-Eseroline O (0.12 g, 0.55 mmol) was dissolved in anhydrous Et₂O (10 mL) and a small piece of Na metal was added. After stirring for about 2 min at room temperature under nitrogen, 2'-methylphenylisocyanate (0.09 g, 0.70 mmol) was added dropwise. After complete addition the solvent was evaporated immediately. The residue was flash chromatographed on a silica gel column (system B) to give (-)-2'-methylphenylcarbamoyl-eseroline as a foam (0.88g, 46%); $[\alpha]_D^{25} -69.6^\circ$ (c=0.5, CHCl₃), CI MS (m/z): 352 (M⁺+1); EI MS (m/z): 351 (M⁺), HR MS (FAB) calcd for (M⁺+1) C₂₁H₂₆N₃O₂ 352.2025, found 352.2020, IR; 3410, 2930, 1745; ¹H NMR 1.46 (s, 3H, C10-CH₃), 1.90-2.12 (m 2H, c3-H), 2.32 (s, 3H, Me-Ph), 2.55 (s, 3H, N1-CH₃), 2.58-2.70 (m, 2H, C2-H₂), 2.91 (s, 3H, N*-CH₃), 4.18 (s, 1H, C9-H), 6.33 (d, J = 8.4, 1H, C7-H), 6.63 (br s, 1H, N-H), 6.85-6.95 (m, 2H, C4-H, C6-H), 7.05 (t, J = 1H, C5'-H), 7.15-7.23 (m, CH, CH₃'-H, C6'-H), 7.85 (br s, 1H, C4'-H).

All other carbamates: (-)-2'-4'-dimethylphenylcarbamoyleseroline, 4'-isopropylcarbamoyleseroline, (-)-2'-ethylphenylcarbamoyleseroline, (-)-2'isopropylphenylcarbamoyleseroline, naphthylcarbamoyl-eseroline, were similarly prepared from (-)-eseroline with the corresponding isocyanates. The important physical data for these compounds are shown below.

EXAMPLE 26(-)-2'-4'-Dimethylphenyl-carbamoyleseroline

The important data is as follows: a foam, $[\alpha]_D^{25} -79.6^\circ$ (c=1, CHCl₃), CI MS (m/z): 366; ¹H NMR 2.28 (s, 3H, C2'-CH₃), 2.29 (s, 3H, C4'-CH₃).

EXAMPLE 27(-)-4'-Isopropylphenylcarbamoyleseroline

The important data is as follows: m.p. ($^{\circ}$ C) 152-153 $[\alpha]_D$ -67.8 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 380 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₃H₃₀N₃O₂, 380.2338; ¹H-NMR: 1.23 (d, J=6.8, 6H, CH(CH₃)₂).

EXAMPLE 28(-)-2'-Ethylphenylcarbamoyleseroline

The important data is as follows: a foam, $[\alpha]_D$ -62.8 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 366 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₂H₂₈N₃O₂, 366.2182; ¹H-NMR: 1.26(t, J=7.5, 3H, -CH₂-CH₃), 2.55-2.78 (m, 4H, C₂-H, -CH₂-CH₃).

EXAMPLE 29(-)-2'-Isopropylphenylcarbamoyleseroline

The important data is as follows: a foam, $[\alpha]_D$ -58.8 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 380 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₃H₂₉N₃O₂, 379.2259; ¹H-NMR: 1.30(d, J=6.8, 6H, -CH-(CH₃)₂).

EXAMPLE 30(-)-1-Naphthylcarbamoyleseroline

The important data is as follows: a foam, $[\alpha]_D$ -62.0 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 388 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₄H₂₆N₃O₂, 388.2025; ¹H-NMR: 7.51(m, 3H), 7.69 (d, J=8.1, 1H), 7.89 (d, J=7.5, 1H), 7.96 (d, J=7.9, 2H).

The related carbamates: (-)-2'methylphenylcarbamoyl-N1-noreseroline, (-)-2',4'-dimethylphenylcarbamoyl-N1-noreseroline, and (-)-4'-isopropylphenylcarbamoyl-N1-noreseroline and

phenylcarbamoyl-N1-noreseroline and (-)-phenyl-carbamoyl-N1-noreseroline were similarly prepared from (-)-(N1)-benzylnoreseroline instead of eseroline by reacting (-)-(N1)-benzylnoreseroline with the corresponding isocyanates. The benzyl protecting group was then removed to yield the noreseroline compound from the benzylnoreseroline compound.

The following example shows the removal of a benzyl protecting group from (-)-2'-methylphenylcarbamoyl-(N1)-benzylnoreseroline to yield compound (-)-2'-methylphenylcarbamoyl-(N1)-noreseroline.

EXAMPLE 31

(-)-2'-Methylphenylcarbamoyl-N1-benzylnoreseroline

(N¹)-Benzylnoreseroline (2.0 g) was dissolved in anhydrous Et₂O (10 ml), and a small piece of Na added. After stirring for 2 minutes at room temperature under nitrogen, 2-methylphenyl isocyanate was added (0.04 g). After stirring for 15 minutes the solvent was evaporated and the residue was chromatographed to give the carbamate as a foam: [α]_D -62.0° (c=0.5, CHCl₃); CI MS (m/z) 428 (M⁺ + 1).

EXAMPLES 32-34

The carbamates of Examples 32-34 belonging to the (-)-N¹-benzylnoreseroline series have been prepared from (-)-N¹-benzylnoreseroline and isocyanates as described in Example 31. The important physical data is as follows.

EXAMPLE 32(-)-2'-4'-Dimethylphenylcarbamoyl-(N1)-benzyl-noreseroline

The important data is as follows: a foam, $[\alpha]_D$ -58.4° (c=0.5, CHCl₃), CI MS (m/z): 442; ¹H NMR 2.28 (2s, 6H), 3.91 (dd, 2H).

EXAMPLE 33(-)-4'-Isopropylphenylcarbamoyl-(N1)-benzyl-noreseroline

The important data is as follows: a foam, $[\alpha]_D$ -44.8° (c=0.5, CHCl₃) CI MS (m/z) 456 (M⁺+1); ¹H-NMR: 1.24 (d, J=7.0, 6H), 3.94 (dd, 2H).

EXAMPLE 34(-)-Phenylcarbamoyl-(N1)-benzylnoreseroline

The important data is as follows: m.p. (°C) 158-159, $[\alpha]_D$ -56.4° (c=0.5, CHCl₃) CI MS (m/z) 414 (M⁺+1); ¹H-NMR: 3.92 (dd, 2H).

EXAMPLE 35(-)-2'-Methylphenylcarbamoyl-N1-noreseroline

(-)-2'-Methylphenylcarbamoyl-N1-benzylnoreseroline (0.09g, 0.21 mmol) from Example 31 was dissolved in MeOH (10 mL), and palladium hydroxide on carbon (7 mg) was added. After hydrogenation under atmospheric pressure for 5 h, the palladium catalyst was filtered through Celite and the solvent was evaporated in vacuo. The residue was chromatographed by preparative TLC (silica gel plate 2000 μm, CH₂Cl₂/10% MeOH) to give (-)-2'-methylphenylcarbamoyl-N1-noreseroline as a foam (0.04g, 56%): $[\alpha]_D$ -62.4° (c = 0.5, CHCl₃), CI MS (m/z): 338 (M⁺+1); EI MS (m/z): 337 (M⁺), HR MS (EI) (M⁺) calcd, for C₂₀H₂₃N₃O₂ 337.1790, found 337.1776 ¹H NMR: 1.42

$C_{20}H_{23}N_3O_2$ 337.1790, found 337.1776 1H NMR: 1.42 (s, 3H, C10-CH₃), 1.70-1.80 (m, 1H, C3-H), 1.95-2.08 (m, 1H, C2-H), 2.29 (s, 3H, C2'-CH₃), 2.70-2.80 (m, 1H, C2-H), 2.81 (s, 1H, N8-CH₃), 3.01-3.10 (ddd, J = 2.5, 2.5, 2.5, 1H, C2-H), 4.51 (s, 1H, C9-H), 6.25 (d, J = 9.0, 1H, C7-H), 6.63 (br s, 1H, N-H), 6.83-6.86 (m, 2H, C4-H, C6-H), 7.02 (t, J = 7.5, 1H, C5'-H), 7.15-7.22 (m, 2H, C3'-H, C6'-H), 7.85 (br s, 1H, C4'-H).

EXAMPLES 36-38

The carbamates of Examples 36-38, belonging to the (-)-N¹-noreseroline series, were prepared from Examples 32-34, belonging to the (-)-N¹-benzylnoreseroline series, by catalytic debenzylation as described in Example 35. The important physical data is as follows.

EXAMPLE 36

(-)-2'-4'-Dimethylphenylcarbamoyl-(N1)-noreseroline

The important data is as follows: a foam, $[\alpha]_D$ -55.4° (c=0.5 CHCl₃), CI MS (m/z): 352; 1H NMR 2.45 (2s, 6H).

EXAMPLE 37

(-)-4'-Isopropylphenylcarbamoyl-(N1)-noreseroline

The important data is as follows: m.p. (°C) 82-84, $[\alpha]_D$ -43.5° (c=0.5, CHCl₃) CI MS (m/z) 366 (M⁺⁺¹); 1H -NMR: 1.23 (d, J=7.0, 6H).

EXAMPLE 38

(-)-Phenylcarbamoyl-(N1)-noreseroline

The important data is as follows: m.p. (°C) 129-131, $[\alpha]_D$ -50.4° (c=0.5, CHCl₃) CI MS (m/z) 324 (M⁺⁺¹); 1H -NMR: 7.25-7.52 (m, 5H).

The following inactive compounds were provided using the above method.

EXAMPLE A'

(-)-4'-methylphenylcarbamoyleseroline

The important data is as follows: m.p. ($^{\circ}$ C) 143-145 $[\alpha]_D$ -74.2 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 352 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₁H₂₆N₃O₂, 352.2025 ¹H-NMR: 2.31 (s, 3H, C4'-CH₃).

EXAMPLE B'

(-)-2',6'-diethylphenylcarbamoyleseroline

The important data is as follows: an oil, $[\alpha]_D$ -36.1 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 394 ($M^+ = 1$); HR MS (FAB): calcd for ($M^+ + 1$) C₂₄H₃₂N₃O₂, 394.2495; ¹H-NMR: 1.24 (t, J=7.4, 6H, 2-CH₂-CH₃), 2.68 (m, 6H, C2-H, 2-CH₂CH₃).

EXAMPLE C'

(-)-2',4',6'-trimethylphenylcarbamoyleseroline

The important data is as follows: a foam, $[\alpha]_D$ -55.8 $^{\circ}$ (c=1, CHCl₃) CI MS (m/z) 380 ($M^+ = 1$); ¹H-NMR: 2.28 (3s, 9H, C2', C4', C6'-CH₃).

The comparative (-)-phenylcarbamoyleseroline compound ((-)-phenserine) was provided as follows.

EXAMPLE D': (-)-Phenylcarbamoyleseroline:

(-)-Eseroline 0 (0.12 g, 0.55 mmol) was dissolved in anhydrous Et₂O (10 mL) and a small piece of Na metal was added. After stirring for about 2 min at room temperature under nitrogen, phenylisocyanate (0.09 g, 0.70 mmol) was added dropwise. After complete addition the solvent was evaporated immediately. The

addition the solvent was evaporated immediately. The residue was flash chromatographed on a silica gel column (system B) to give (-)-phenylcarbamoyleseroline mp(°C) 142-143 (0.88g, 46%); $[\alpha]_D^{25} -74.2^\circ$ ($c=1$, CHCl_3), CI MS (m/z): 338; ^1H NMR 7.01 (t, $J=7.4$, 1H, C4'-H), 7.22 (t, $J=7.4$, 2H, C3'-H, C5'-H), 7.34 (d, $J=7.4$, 2H, C2'-H, C6'-H). The common name for this compound is (-)-phenserine.

The compound numbers in Tables 3 and 5 correspond to one another and to the above Examples. Comparative Example D' is (-)-Phenserine.

Table 5 below lists the important physical data for compounds according to the invention, which correspond to the compound numbers in Table 3.

Table 5

IC₅₀ Values of Phenylcarbamates of (-)-Eseroline, (-)-Physovenol, and (-)-N¹-Noreseroline vs. Human Erythrocyte AChE and Human Plasma BChE

No.	AChE	IC ₅₀ [nmol]		BChE
		IC ₅₀	[nmol]	
Biological standards				
A	physostigmine	27.9	± 2.4	16.0 ± 2.9
B	N'norphysostigmine	21.0	± 1.0	2.0 ± 1.0
C	physovenine	27.1	± 0.8	3.7 ± 1.4
D'	phenserine	24.0	± 6.0	1300 ± 85.0
Examples				
Ex. 25		10.3	± 1.6	1948.5 ± 245.5
Ex. 26		13.6	± 1.0	1817.0 ± 558.5
Ex. 27		758.2	± 21.2	51.3 ± 0.9
Ex. 28		9.7	± 0.7	2916.0 ± 537.0
Ex. 29		15.5	± 1.3	647.8 ± 46.2
Ex. 30		16.1	± 1.0	1832.0 ± 35.5
Ex. 31		not tested		
Ex. 32		not tested		
Ex. 33		> 10,000		45.3 ± 4.6
Ex. 34		not tested		
Ex. 35		17.0	± 0.2	2165.0 ± 85.0
Ex. 36		17.3	± 1.2	1139.0 ± 26.0
Ex. 37		322.4	± 3.7	8.3 ± 1.0
Ex. 38		13.8	± 0.7	612.0 ± 0.4
Inactive Compounds				
Ex. A'		139.2	± 3.7	251.1 ± 8.6
Ex. B'		1493.7	± 49.8	1073.5 ± 48.0
Ex. C'		1291.9	± 73.8	1817.0 ± 885.0

In vitro assay of human anti-AChE and -BChE activity,
 IC_{50}

A classical enzyme inhibition assay was undertaken to quantitate the activity of the derivatives against AChE and BChE. Anti-cholinesterase activity was determined against human erythrocyte AChE and plasma BChE in 0.1 M Na_3PO_4 buffer (pH 8.0) using the spectrophotometric method of Ellman et al. (Biochem. Pharmacol. 7:88, 1961). Freshly collected plasma was diluted 1:125 with 0.1 M Na_3PO_4 (pH 7.4) and lysed erythrocytes similarly diluted to 1:200. Acetyl-B-methylthiocholine (0.5 mM) and s-butyrylthiocholine (0.5 mM) were used as specific substrates for AChE and BChE, respectively, 25 μ l of substrate and 25 μ l of enzyme in a total volume of 0.75 ml.

Physostigmine derivatives, diluted in half log-intervals to a concentration range of between $1 \times 10^{-5} M$ and $3 \times 10^{-10} M$, were preincubated with enzyme (30 min at 21°C) prior to addition of substrates. Following incubation (30 min at 37°C), production of a yellow thionitrobenzoate anion was measured with a spectrophotometer set to 412 nm wavelength. Nonspecific substrate hydrolysis was determined under conditions of complete enzyme inhibition (by addition of physostigmine $1 \times 10^{-5} M$), and the associated change in absorbance subtracted from that observed with the test compounds. Finally, the activity of each compound was assessed alongside that of physostigmine, as an external standard, whose activity has been previously reported (Atack et al., J. Pharm. Expl. Ther. 249:294, 1989).

The AChE and BChE activity of each compound was expressed as an IC₅₀, which is defined as the concentration in nmol required to inhibit 50% of enzyme activity (calculated as described by Atack et al., J. Pharm. Expl. Ther. 249:294, 1989)).

In vivo duration of activity studies

Catheters, filled with heparinized saline, were tied into the right femoral vein and artery of anesthetized male rats, which then were restrained in a plaster cast and allowed to recover from anesthesia in a temperature-controlled enclosure. Plasma samples were withdrawn to quantitate untreated levels of AChE activity. At 90 min. after surgery, hexamethonium bromide (5 mg/kg, i.p.) was administered, followed by atropine methylbromide (4 mg/kg, s.c.) 10 min. later. These quaternary nicotinic and muscarinic antagonists, do not enter brain and inhibit peripheral cholinergic overdrive associated with cholinesterase inhibition, which may be deleterious to the animal. Two hours after surgery, either (i) physostigmine, (ii) physostigmine derivatives, or (III) THA was administered i.v. Plasma samples were removed at intervals between 2 min. and 8 hr., immediately frozen to -70°C and then assayed for cholinesterase inhibition. AChE inhibition was measured as described above, with necessary modifications required for quantitation from rat plasma.

All drugs were formulated in a manner consistent with i.v. administration. Specifically, drugs were dissolved in Tween 80/EtOH (3:1, V:V), approximately 100 µl, and then were diluted in excess of 1:9 (V:V) with isotonic saline. The use of Tween 80/EtOH did not

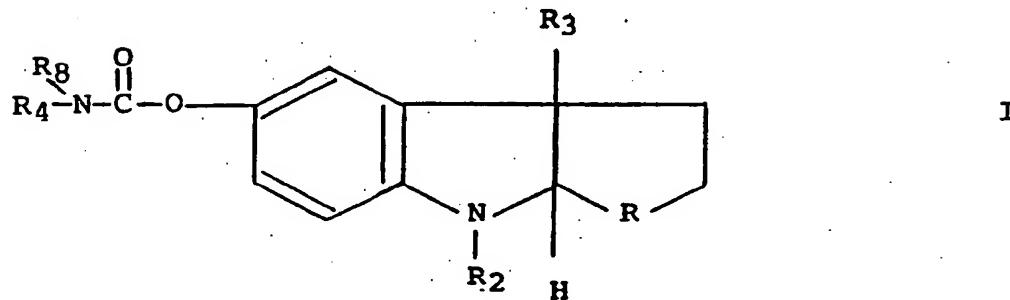
affect either AChE or BChE inhibitory activity of compounds in in vitro studies (Yu et al., *Helv. Chim. Acta* 74, pages 761-766, (1991)). Doses were determined in prior studies involving the measurement of rectal temperature and tremor; two centrally-mediated actions of cholinesterase inhibitors and cholinergic agonists.

Figure 1 demonstrates the in vivo inhibition of the enzyme acetylcholinesterase (AChE) by physostigmine and its 2',4'-dimethylphenyl carbamate derivative, i.e., the time-dependent activity of these cholinesterase inhibitors in rats. As predicted from the in vitro IC₅₀ studies, physostigmine and the substituted phenyl carbamates to which this patent relates (which are represented in this case by 2',4'-dimethylphenyl physostigmine) possess excellent in vivo cholinesterase inhibitory properties. However, the duration of enzyme inhibition is short following an intravenous bolus of physostigmine. Whereas a peak inhibition of 46% occurred within 2 minutes of administration, this rapidly declined to 25% by 15 minutes and was negligible at one hour. An equal dose of the 2',4'-methylphenyl carbamate resulted in immediate 60% AChE inhibition at 2 minutes. This was maintained at a steady level for 2 hours and then slowly declined to 36% inhibition at 8 hours. The high activity, specificity and persistence of 2',4'-dimethylphenyl physostigmine, which is achieved without side-effects or toxicity, is surprising and supports the contention that these compounds represent a class of potent, new and selective cholinesterase inhibitors.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without departing from the generic concept and therefore such adaptations are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description only and not of limitation.

Claims

1. A compound according to the Formula I



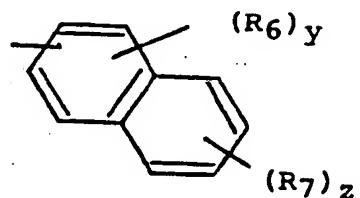
wherein R is -0- and

R₂ and R₃ are independently selected from H or-C₁-C₁₀-alkyl;

R₄ is



or



wherein

R₅, R₆, and R₇ are independently selected from H, halogen or -C₁-C₁₀-alkyl,

x is 0 or an integer from 1-5,

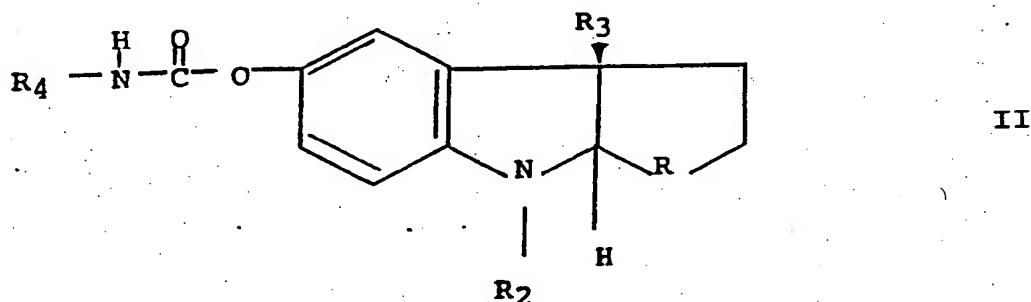
y is 0 or an integer from 1-3,

z is 0 or an integer from 1-4; and R₈ is H or -C₁-C₁₀-alkyl;

including isomeric forms, and

pharmacologically acceptable salts.

2. A compound according to claim 1, Formula I and having the Formula II



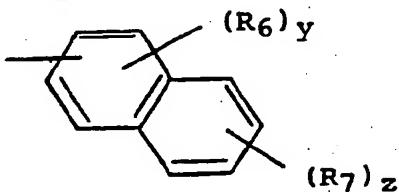
wherein R is -O- and

R₂ and R₃ are independently selected from H, halogen or -C₁-C₁₀-alkyl; and

R₄ is



or



wherein

R₅, R₆ and R₇ are independently selected from H or -C₁-C₁₀-alkyl,

x is 0 or an integer from 1-5,

y is 0 or an integer from 1-3, and

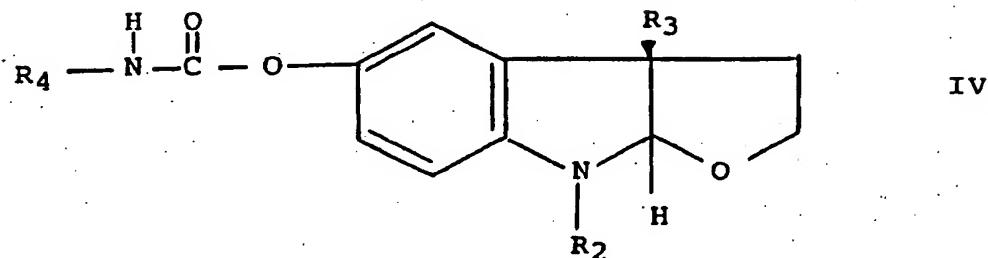
z is 0 or an integer from 1-4;

including isomeric forms, and

pharmacologically acceptable salts.

SUBSTITUTE SHEET

3. A compound according to claim 1 having the formula IV wherein

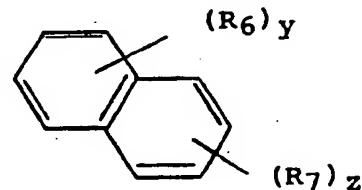


R_2 and R_3 are independently selected from H or $-C_1-C_{10}$ -alkyl; and

R_4 is



or



wherein

R_5 , R_6 , and R_7 are independently selected from H, halogen or $-C_1-C_{10}$ -alkyl,

x is 0 or an integer from 1-5,

y is 0 or an integer from 1-3, and

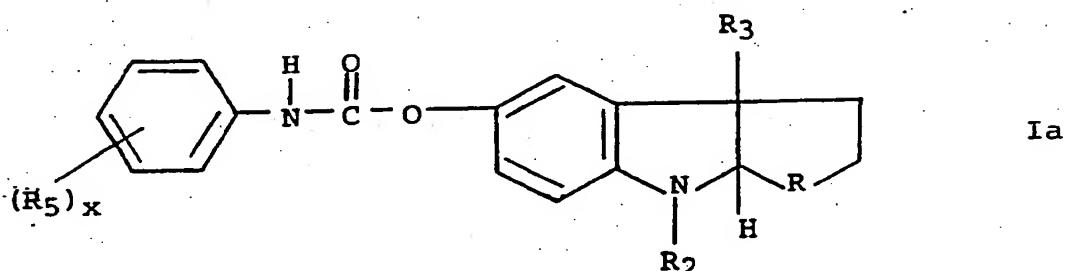
z is 0 or an integer from 1-4;

including isomeric forms, and pharmacologically acceptable salts.

4. A compound according to claim 3, wherein R_3 is a methyl group.

5. A compound according to claim 3, wherein R_2 and R_3 both represent a methyl group.

6. A compound according to claim 1, having the formula Ia



wherein

R is -O- and

R₂ and R₃ are independently selected from H or -C₁-C₁₀-alkyl; and

R₅ is independently selected from H, halogen or -C₁-C₁₀-alkyl and x is 0 or an integer from 1-5 including isomeric forms, and

pharmacologically acceptable salts.

7. A compound according to claim 6, where x is 1 or 2 and R₅ is in the ortho and/or para position.

8. A compound according to claim 6, wherein R₅ is independently selected from the group consisting of CHLORO, -CH₃, -CH₂-CH₃, and -CH(-CH₃)₂ and x is an integer from 1-5.

9. A pharmaceutical composition comprising the pharmaceutically effective amount of a compound according to claim 1 and a carrier.

10. A method for treating cholinergic disorders comprising administration of an effective amount of a compound according to claim 1 to a mammal in need of such treatment.

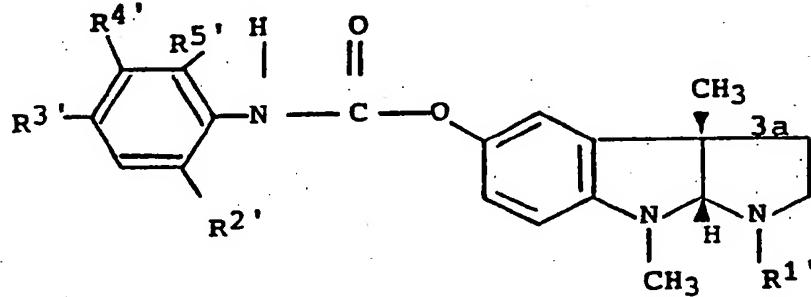
11. A method according to claim 10, wherein the cholinergic disorder is selected from the group consisting of glaucoma, Myasthenia Gravis, Alzheimer's disease.

12. A method for inhibiting acetylcholinesterase activity comprising administering an effective amount of a compound according to claim 1 to a mammal in need thereof.

13. A method for inhibiting butyrylcholinesterase activity in a mammal comprising administering an effective amount of a compound according to claim 1.

14. A method for treating organophosphate poisoning in a mammal comprising administering an effective amount of a compound according to claim 1.

15. A compound according to the formula



wherein R^{1'} is H, a -CH₃ group or a benzyl group;
R^{2'} is straight or branched chained C₁-C₁₀ alkyl;
R^{3'} is H or a straight or branched C₁-C₁₀ alkyl;
and
R^{4'} and R^{5'} are independently hydrogen or R⁴' and
R⁵' taken together along with the carbon atoms to which
they are attached form a 6-membered aromatic
carbocyclic ring;
and pharmaceutically acceptable salts.

16. A compound selected from the group consisting
of
(-)-2'-methylphenylcarbamoyleseroline,
(-)-2'-4'-dimethylphenylcarbamoyleseroline,
5 (-)-4'-isopropylcarbamoyleseroline,
(-)-2'-ethylphenylcarbamoyleseroline,
(-)-2'-isopropylphenylcarbamoyleseroline,
(-)-naphthylcarbamoyleseroline,
(-)-2'methylphenylcarbamoyl-N1-noreseroline,
10 (-)-2',4'-dimethylphenylcarbamoyl-N1-noreseroline,
(-)-4'-isopropylphenylcarbamoyl-N1-noreseroline,
(-)-phenylcarbamoyl-N1-noreseroline,
(-)-2'methylphenylcarbamoyl-N1-benzyl-noreseroline,
15 (-)-2',4'-dimethylphenylcarbamoyl-N1-benzyl-
noreseroline,
(-)-4'-isopropylphenylcarbamoyl-N1-benzyl-
noreseroline,
and (-)-phenylcarbamoyl-N1-benzyl-noreseroline.

17. A compound according to claim 15, wherein R^{1'}
is hydrogen.

18. A compound according to claim 1, wherein R¹ is -CH₃.

19. A compound according to claim 15, wherein R¹ is a benzyl group.

20. A compound according to claim 15, wherein R¹ is H or a -CH₃ group; R² is straight or branched chained C₁-C₁₀-alkyl;

5 R³ is H or straight or branched chained C₁-C₁₀ alkyl; and

R⁴ and R⁵ are independently hydrogen or R⁴ and R⁵ taken together along with the carbon atoms to which they are attached form a 6-membered aromatic carbocyclic ring;

10 and pharmaceutically acceptable salts.

21. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound according to claim 15 and a carrier.

22. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound according to claim 16 and a carrier.

23. A method for treating cholinergic disorders comprising administration of an effective amount of a compound according to claim 15 to a mammal in need of such treatment.

24. A method according to claim 23, wherein the cholinergic disorder is selected from the group consisting of glaucoma, Myasthenia Gravis, Alzheimer's disease.

24. A method for inhibiting acetylcholinesterase activity comprising administering an effective amount of a compound according to claim 15 to a mammal in need thereof.

25. A method for inhibiting acetylcholinesterase activity comprising transdermally administering an effective amount of a compound according to claim 15 to a mammal in need thereof.

26. A method for inhibiting butyrylcholinesterase activity comprising administering an effective amount of a compound according to claim 15 to a mammal.

27. A method for treating organophosphate poisoning in a mammal comprising administering an effective amount of a compound according to claim 15.

28. A method for treating cholinergic disorders comprising administration of an effective amount of a compound according to claim 16 to a mammal in need of such treatment.

29. A method according to claim 28, wherein the cholinergic disorder is selected from the group consisting of glaucoma, Myasthenia Gravis, Alzheimer's disease.

30. A method for inhibiting butyrylcholinesterase activity comprising administering an effective amount of a compound according to claim 16 to a mammal.

31. A method for inhibiting butyrylcholinesterase activity comprising transdermally administering an effective amount of a compound according to claim 16 to a mammal in need thereof.

32. A method for treating organophosphate poisoning in a mammal comprising administering an effective amount according to claim 16.

1/1

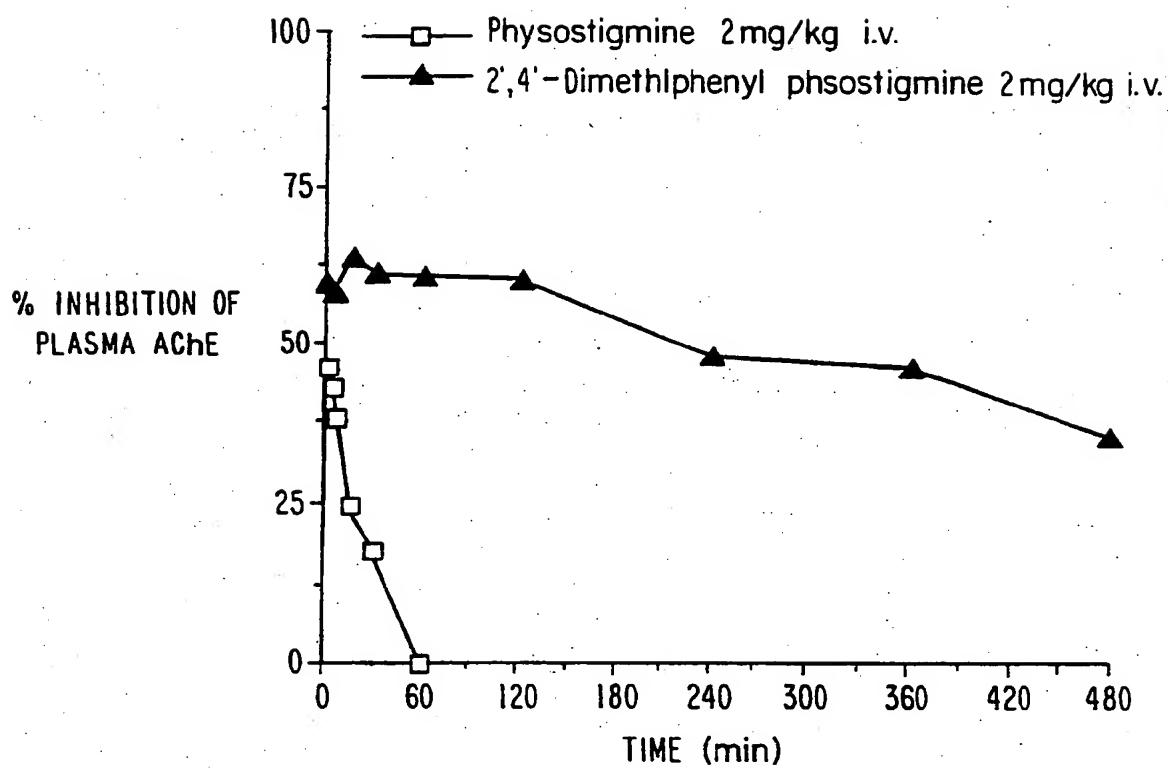


Figure 1

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/08228

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.C1. 5 C07D487/04; C07D491/048; A61K31/40; // (C07D487/04, 209:00, 209:00) (C07D491/048, 307:00, 209:00)

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System	Classification Symbols
Int.C1. 5	C07D ; A61K

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched⁸III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	HELVETICA CHIMICA ACTA vol. 74, no. 4, 19 June 1991, BASEL CH pages 761 - 766 Q.-S. YU ET AL. 'Physovenines: Efficient synthesis of (-)- and (+)-physovenine and synthesis of carbamate analogues of (-)-physovenine. Anticholinesterase activity and analgesic properties of optically active physovenines' see page 761, abstract; page 762, compounds 9a,10a; page 763, Table 1 ---	1,10,12, 13
X	EP,A,0 154 864 (CONSIGLIO NAZIONALE DELLE RICERCHE) 18 September 1985 see page 2, line 1 - line 12; claims 1,5; example 4 ---	15,23,24 -/-

¹⁰ Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

02 DECEMBER 1992

Date of Mailing of this International Search Report

28.01.93

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

VOYIAZOGLOU D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category ^a	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 253 372 (HOECHST-ROUSSEL) 20 January 1988 see claims 1,12; example 26 -----	15,23,24

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9208228
SA 65478**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 02/12/92

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0154864	18-09-85	EP-A-	0354594	14-02-90
		JP-B-	3054952	21-08-91
		JP-A-	60208982	21-10-85
		US-A-	4831155	16-05-89
EP-A-0253372	20-01-88	US-A-	4791107	13-12-88
		AU-B-	612583	18-07-91
		AU-A-	7566887	21-01-88
		JP-A-	63023881	01-02-88

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
9 October 2003 (09.10.2003)

PCT

(10) International Publication Number
WO 03/083071 A3

(51) International Patent Classification⁷: **A61K 39/395, C12N 5/00, 5/02**

(21) International Application Number: **PCT/US03/09459**

(22) International Filing Date: **26 March 2003 (26.03.2003)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
60/367,902 26 March 2002 (26.03.2002) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
24 December 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/083071 A3

(54) Title: **DIABETES-RELATED IMMUNOGLOBULIN DERIVED PROTEINS, COMPOSITIONS, METHODS AND USES**

(57) Abstract: The present invention relates to at least one novel diabetes related human Ig derived protein or specified portion or variant, including isolated nucleic acids that encode at least one diabetes related Ig derived protein or specified portion or variant, diabetes related Ig derived protein or specified portion or variants, vectors, host cells, transgenic animals or plants, and methods of making and using thereof, including therapeutic compositions, methods and devices.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/09459

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 39/395; C12N 5/00, 5/02
US CL : 424/130.1; 514/866; 435/387.1, 387.9

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/130.1; 514/866; 435/387.1, 387.9

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, Medline, Caplus, Biosis, Embase, Scisearch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	RABINOVITCH, A., An Update on Cytokines in the Pathogenesis of Insulin-dependent Diabetes Mellitus. <i>Diabetes Metab. Rev.</i> June 1998, Vol. 14, No. 2, pages 129-151, see entire document.	1-22
Y	ANDRE-SCHMUTZ, I., et al., Cellular and molecular changes accompanying the progression from insulin to diabetes. <i>Eur. J. Immunol.</i> January 1999, Vol. 29, No. 1, pages 245-255, see entire document.	1-22
Y	NICOLETTI, F., et al., Prevention of Spontaneous Autoimmune Diabetes in Diabetes-Prone BB Rats by Prophylactic Treatment with Antirat Interferon-gamma Antibody. <i>Endocrinology</i> . 1997, Vol. 138, No. 1, pages 281-288, see entire document.	1-22
Y	FUJIHIRA, K., et al., Suppression and Acceleration of Autoimmune Diabetes by Neutralization of Endogenous Interleukin-12 in NOD Mice. <i>Diabetes</i> . December 2000, Vol. 49, pages 1998-2006, see entire document.	1-22

Further documents are listed in the continuation of Box C.



See patent family annex.

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"A"	document defining the general state of the art which is not considered to be of particular relevance
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"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search

13 August 2003 (13.08.2003)

Date of mailing of the international search report

24 OCT 2003

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